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FILE 'CAPLUS' ENTERED AT 15:27:10 ON 26 JUL 2003
L1 38 NMR (S) (MULTIVARIAT? OR PROTEOMIC? OR SCORE? OR PATTERN?) (S) (MEDICIN? OR PLANT? OR DRUG?)

L1 ANSWER 1 OF 38 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2003:527133 CAPLUS
TITLE: "The opportunities and challenges of personalized genome-based molecular therapies for cancer: targets, technologies, and molecular chaperones"
AUTHOR(S): *Workman, Paul*
CORPORATE SOURCE: Cancer Research UK Centre for Cancer Therapeutics, Institute of Cancer Research, Sutton, Surrey, SN2 5NG
SOURCE: **Cancer Chemotherapy and Pharmacology (2003), 52(s01), 45-56**
CODEN: CCPHDZ; ISSN: 0344-5704
PUBLISHER: Springer-Verlag
DOCUMENT TYPE: Journal
LANGUAGE: English
AB There are now unprecedented opportunities for the development of improved drugs for cancer treatment. Following on from the Human Genome Project, the Cancer Genome Project and related activities will define most of the genes in the majority of common human cancers over the next 5 yr. This will provide the opportunity to develop a range of drugs targeted to the precise mol. abnormalities that drive various human cancers and opens up the possibility of personalized therapies targeted to the mol. pathol. and genomics of individual patients and their malignancies. The new mol. therapies should be more effective and have less-severe side effects than cytotoxic agents. To develop the new generation of mol. cancer therapeutics as rapidly as possible, it is essential to harness the power of a range of new technologies. These include: genomic and proteomic methodologies (particularly gene expression microarrays); robotic high-throughput screening of diverse compd. collections, together within silico and fragment-based screening techniques; new structural biol. methods for rational drug design (esp. high-throughput X-ray crystallog. and NMR); and advanced chem. technologies, including combinatorial and parallel synthesis. Two major challenges to cancer drug discovery are: (1) the ability to convert potent and selective lead compds. with activity by the desired mechanism on tumor cells in culture into agents with robust, drug-like properties, particularly in terms of pharmacokinetic and metabolic properties; and (2) the development of validated pharmacodynamic endpoints and mol. markers of drug response, ideally using noninvasive imaging technologies. The use of various new technologies will be exemplified. A major conceptual and practical issue facing the development and use of the new mol. cancer therapeutics is whether a single drug that targets one of a series of key mol. abnormalities in a particular cancer (e.g. BRAF) will be sufficient on its own to deliver clin. benefit ("house of cards" and tumor addiction models). The alternative scenario is that it will require either a combination of agents or a class of drug that has downstream effects on a range of oncogenic targets. Inhibitors of the heat-shock protein (HSP) 90 mol. chaperone are of particular interest in the latter regard, because they offer the potential of inhibiting multiple oncogenic pathways and

simultaneous blockade of all six "hallmark traits" of cancer through direct interaction with a single mol. drug target. The first-in-class HSP90 inhibitor 17AAG exhibited good activity in animal models and is now showing evidence of mol. and clin. activity in ongoing clin. trials. Novel HSP90 inhibitors are also being sought. The development of HSP90 inhibitors is used to exemplify the application of new technologies in drug discovery against a novel mol. target, and in particular the need for innovative pharmacodynamic endpoints is emphasized as an essential component of hypothesis-testing clin. trials.

L1 ANSWER 2 OF 38 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2003:103650 CAPLUS
DOCUMENT NUMBER: 139:48396
TITLE: "Metabolomic analysis of the consequences of cadmium exposure in Silene cucubalus cell cultures via 1H NMR spectroscopy and chemometrics"
AUTHOR(S): *Bailey, Nigel J. C.; Oven, Matjaz; Holmes, Elaine; Nicholson, Jeremy K.; Zenk, Meinhart H.*
CORPORATE SOURCE: Technology and Medicine, Imperial College of Science, Biomedical Sciences Division, Biological Chemistry, University of London, London, SW7 2AZ, UK
SOURCE: **Phytochemistry (Elsevier) (2003), 62(6), 851-858**
CODEN: PYTCAS; ISSN: 0031-9422
PUBLISHER: Elsevier Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Several essential and non-essential metals (typically those from periods 4, 5 and 6 in groups 11-15 in the periodic table) are commonly detoxified in higher plants by complexation with phytochelatin. The genetic and gross metabolic basis of metal tolerance in plants is, however, poorly understood. Here, we have analyzed plant cell exts. using 1H NMR spectroscopy combined with multivariate statistical anal. of the data to investigate the biochem. consequences of Cd2+ exposure in Silene cucubalus cell cultures. Principal components anal. of 1H NMR spectra showed clear discrimination between control and Cd2+ dosed groups, demonstrating the metabolic effects of Cd2+ and thus allowing the identification of increases in malic acid and acetate, and decreases in glutamine and branched chain amino acids as consequences of Cd2+ exposure. This work shows the value of NMR-based metabolomic approaches to the detn. of biochem. effects of pollutants in naturally selected populations.
REFERENCE COUNT: 25

L1 ANSWER 3 OF 38 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2003:103648 CAPLUS
TITLE: "Metabonomics classifies pathways affected by bioactive compounds. Artificial neural network classification of NMR spectra of plant extracts"
AUTHOR(S): *Ott, Karl-Heinz; Aranibar, Nelly; Singh, Bijay; Stockton, Gerald W.*
CORPORATE SOURCE: BASF Agro Research, Princeton, NJ, 08543, USA
SOURCE: **Phytochemistry (Elsevier) (2003), 62(6), 971-985**
CODEN: PYTCAS; ISSN: 0031-9422

PUBLISHER: Elsevier Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The biochem. mode-of-action (MOA) for herbicides and other bioactive compds. can be rapidly and simultaneously classified by automated pattern recognition of the metabonome that is embodied in the 1H NMR spectrum of a crude plant ext. The ca. 300 herbicides that are used in agriculture today affect less than 30 different biochem. pathways. In this report, 19 of the most interesting MOAs were automatically classified. Corn (Zea mays) plants were treated with various herbicides such as imazethapyr, glyphosate, sethoxydim, and diuron, which represent various biochem. modes-of-action such as inhibition of specific enzymes (acetohydroxy acid synthase [AHAS], protoporphyrin IX oxidase [PROTOX], 5-enolpyruvylshikimate-3-phosphate synthase [EPSPS], acetyl CoA carboxylase [ACC-ase], etc.), or protein complexes (photosystems I and II), or major biol. process such as oxidative phosphorylation, auxin transport, microtubule growth, and mitosis. Crude isolates from the treated plants were subjected to 1H NMR spectroscopy, and the spectra were classified by artificial neural network anal. to discriminate the herbicide modes-of-action. We demonstrate the use and refinement of the method, and present cross-validated assignments for the metabolite NMR profiles of over 400 plant isolates. The MOA screen also recognizes when a new mode-of-action is present, which is considered extremely important for the herbicide discovery process, and can be used to study deviations in the metab. of compds. from a chem. synthesis program. The combination of NMR metabolite profiling and neural network classification is expected to be similarly relevant to other metabonomic profiling applications, such as in drug discovery.
REFERENCE COUNT: 37

L1 ANSWER 4 OF 38 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2003:103641 CAPLUS
TITLE: "Assessment of 1H NMR spectroscopy and multivariate analysis as a technique for metabolite fingerprinting of Arabidopsis thaliana"
AUTHOR(S): *Ward, Jane L.; Harris, Cassandra; Lewis, Jennie; Beale, Michael H.*
CORPORATE SOURCE: Department of Agricultural Sciences, IACR-Long Ashton Research Station, University of Bristol, Bristol, BS41 9AF, UK
SOURCE: **Phytochemistry (Elsevier) (2003), 62(6), 949-957**
CODEN: PYTCAS; ISSN: 0031-9422
PUBLISHER: Elsevier Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB An approach to metabolite fingerprinting of crude plant exts. that utilizes 1H NMR (NMR) spectroscopy and multivariate statistics has been tested. Using ecotypes of Arabidopsis thaliana as exptl. material, a method has been developed for the rapid anal. of unfractionated polar plant exts., enabling the creation of reproducible metabolite fingerprints. These fingerprints could be readily stored and compared by a variety of chemometric methods. Comparison by principal component anal. using SIMCA-P allowed the generation of residual NMR spectra of the compds. that contributed significantly to the differences between samples. From these plots, conclusions were

drawn with respect to the identity and relative levels of metabolites differing between samples.
REFERENCE COUNT: 16

L1 ANSWER 5 OF 38 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2002:700443 CAPLUS
DOCUMENT NUMBER: 139:12412
TITLE: "Multi-component metabolic classification of commercial feverfew preparations via high-field 1H-NMR spectroscopy and chemometrics"
AUTHOR(S): *Bailey, Nigel J. C.; Sampson, Julia; Hylands, Peter J.; Nicholson, Jeremy K.; Holmes, Elaine*
CORPORATE SOURCE: Biological Chemistry, Biomedical Sciences Division, Imperial College of Science, Technology and Medicine, University of London, London, SW7 2AZ, UK
SOURCE: **Planta Medica (2002), 68(8), 734-738**
CODEN: PLMEAA; ISSN: 0032-0943
PUBLISHER: Georg Thieme Verlag
DOCUMENT TYPE: Journal
LANGUAGE: English
AB There is increasing interest in evaluating the clin. efficacy of herbal medicines. However, there are significant anal. problems assocd. with quality control and the measurement of the overall compn. of such complex, multi-component mixts. as normally required in the pharmaceutical industry. Here we describe a novel NMR spectroscopic and pattern recognition anal. approach to investigate compn. and variability of a commonly used herbal medicine. 1H-NMR spectroscopy (600 MHz) and principal component anal. (PCA) were used to discriminate between batches of 14 com. available feverfew samples based on multi-component metabolite profiles. Two of the batches were significantly different from the other 12. The 12 remaining classes could be classified into discrete groups by PCA on the basis of minor differences in overall chem. compn. NMR based pattern recognition anal. of exts. was superior to PR anal. of HPLC traces of the same mixts. This work indicates the potential value of NMR combined with PCA for the characterization of complex natural product mixts., and the discrimination of samples contg. allegedly identical ingredients.
REFERENCE COUNT: 19

L1 ANSWER 6 OF 38 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2002:685562 CAPLUS
DOCUMENT NUMBER: 138:280546
TITLE: "NMR-based metabolic analysis - new technique in drug research and development"
AUTHOR(S): *Sidelmann, Ulla Grove*
CORPORATE SOURCE: Applied Trinomics, Novo Nordisk, Den.
SOURCE: **Dansk Kemi (2002), 83(8), 23-24, 26-27**
CODEN: DAKEAT; ISSN: 0011-6335
PUBLISHER: TechMedia
DOCUMENT TYPE: Journal; General Review

LANGUAGE: Danish
AB A review. NMR spectroscopy combined with multivariate data anal. can provide quant. and qual. information concerning endogenous metabolites in biol. samples. This can be applied in toxicol. and pharmacol.

L1 ANSWER 7 OF 38 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2002:590584 CAPLUS
DOCUMENT NUMBER: 137:284140
TITLE: NMR-Based Metabonomic Studies on the Biochemical Effects of Commonly Used Drug Carrier Vehicles in the Rat
AUTHOR(S): Beckwith-Hall, Bridgette M.; Holmes, Elaine; Lindon, John C.; Gounarides, John; Vickers, Alison; Shapiro, Michael; Nicholson, Jeremy K.
CORPORATE SOURCE: Biological Chemistry Biomedical Sciences Division, Imperial College of Science Technology and Medicine, London, SW7 2AZ, UK
SOURCE: Chemical Research in Toxicology (2002), 15(9), 1136-1141
CODEN: CRTOEC; ISSN: 0893-228X
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The biochem. effects of a series of commonly used drug carrier vehicles were investigated using 1H NMR spectroscopic and pattern recognition based metabonomic anal. Animals were treated by oral gavage with six dosage vehicles: 0.5% (w/v) sodium CM-cellulose/0.2% (vol./vol.) tween; microemulsion (consisting of propylene glycol, ethanol, cremophor, and corn oil glycerides); labrafil [consisting of poly(ethylene glycol) 300 esterified with oleic acid] (30%)/corn oil (70%); 0.1 M sodium phosphate buffered water; poly(ethylene glycol) 300 and 0.5% methocel. Urine samples (n = 7) collected over a 96 h period post administration were analyzed using 600 MHz 1H NMR spectroscopy, and principal components anal. of the spectral data was used to analyze these data. Of the six vehicles studied, three (labrafil/corn oil, PEG 300 and microemulsion) gave rise to strong vehicle-related signals in the 1H NMR spectra of urine and were, therefore, deemed to be less suitable for NMR-based toxicity studies. To investigate any biochem. consequences of vehicle dosing, PCA was used to analyze spectral regions that did not contain vehicle-related signals, i.e., the NMR-detectable endogenous metabolite profile. PEG 300 and labrafil/corn oil induced changes in the biochem. compn. of urine including increased concns. of dicarboxylic acids, creatinine, taurine, and sugars, indicating that these vehicles were bioactive in their own right and that this might confound interpretation of biochem. effects of weakly toxic drugs dosed in these carriers. This study shows the importance of selecting appropriate vehicles for NMR-based metabonomic studies with a view to minimizing the possibility of vehicle resonances obscuring endogenous compd. peaks. Furthermore, we have shown that at least two of the commonly used drug carrier vehicles caused metabolic perturbations in the urine profile. These alterations in the biochem. profile reflect vehicle-induced changes in the physiol. status of the organism that may obscure the pharmacol. or toxicol. effects of drugs.
REFERENCE COUNT: 25

L1 ANSWER 8 OF 38 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2002:123345 CAPLUS
DOCUMENT NUMBER: 136:164279
TITLE: "Quality control and standardization of tobacco by means of NMR and pattern recognition"
INVENTOR(S): *Hylands, Peter John*
PATENT ASSIGNEE(S): High Value Horticulture Limited, UK
SOURCE: PCT Int. Appl., 38 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002012872	A1	20020214	WO 2001-GB3540	20010806
AU 2001076526	A5	20020218	AU 2001-76526	20010806
EP 1307730	A1	20030507	EP 2001-954180	20010806

PRIORITY APPLN. INFO.: GB 2000-19264 A 20000804
WO 2001-GB3540 W 20010806

AB A process for establishing a std. specification for a tobacco plant material, comprises: (i) prepg. a test soln. or test ext. of a sample of the tobacco plant material which is known to possess the or each property required for the std.; (ii) submitting the said soln. or ext. to anal. by a combination of NMR spectroscopy and a computer-based pattern recognition technique; (iii) obtaining results from the anal. methods used in step (ii); and (iv) establishing a std. specification for the said tobacco plant material on the basis of the results obtained in step (iii). Candidate samples of the tobacco plant material may subsequently be tested for compliance with the std. They can be accepted or rejected depending on whether they five anal. results which fall within or outside either part or all of the specification established in step (iv). Thus, NMR spectroscopy combined principal component anal. successfully discriminated between tobacco from cigarettes sold under different brand names.
REFERENCE COUNT: 3

L1 ANSWER 9 OF 38 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2001:885049 CAPLUS
DOCUMENT NUMBER: 136:95486
TITLE: "Screening with NMR"
AUTHOR(S): *Bradley, David*
CORPORATE SOURCE: Washington DC, USA
SOURCE: **Modern Drug Discovery (2001), 4(11), 28-30,32,34**
CODEN: MDDIFT; ISSN: 1099-8209
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
AB A review discussing the advances in NMR techniques towards successful use in combinatorial drug anal., drug fragments screening and drug metab. anal.
REFERENCE COUNT: 9

L1 ANSWER 10 OF 38 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2001:829171 CAPLUS
Correction of: 1997:3670
DOCUMENT NUMBER: 135:330642
Correction of: 126:59027
TITLE: "Multivariate statistical analysis of two-dimensional NMR data to differentiate grapevine cultivars and clones"
AUTHOR(S): *Vorveille, Laurence; Vercauteren, Joseph; Rutledge, Douglas N.*
CORPORATE SOURCE: Lab. Pharmacognosie, Univ. Bordeaux II, Bordeaux, 33076, Fr.
SOURCE: **Food Chemistry (1996), 57(3), 441-450**
CODEN: FOCHDJ; ISSN: 0308-8146
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Multivariate statistical methods were applied on two-dimensional NMR data (1H-13C) of polyphenol exts. from grapevine clones harvested in the Bordeaux region. An anal. of variance detected the most discriminating NMR spectral correlation vols. which were then used to perform principal component, hierarchical clustering and discriminant analyses. The results showed that clones are divided into three groups according to the cultivar and they can be differentiated inside each cultivar. This method was applied to polyphenol exts. from grape seeds and leaves. Two-dimensional NMR is the only anal. tool which can differentiate grapevine clones. It could thus be used to check the identity of grapevine species, cultivars or clones as well as any other plants which produce polyphenols.

L1 ANSWER 11 OF 38 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2001:9445 CAPLUS
DOCUMENT NUMBER: 134:187790
TITLE: "A metabonomic approach to the investigation of drug-induced phospholipidosis: an NMR spectroscopy and pattern recognition study"
AUTHOR(S): *Nicholls, Andrew W.; Nicholson, Jeremy K.; Haselden, John N.; Waterfield, Catherine J.*
CORPORATE SOURCE: Biological Chemistry, Biomedical Sciences Division, Imperial College of Science, Technology and Medicine, London, SW7 2AZ, UK
SOURCE: **Biomarkers (2000), 5(6), 410-423**
CODEN: BIOMFA; ISSN: 1354-750X
PUBLISHER: Taylor & Francis Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB 1H NMR spectroscopy of urine and pattern recognition anal. have been used to study the metabolic perturbations caused following dosing of five novel drug candidates, two of which (GWA, GWB) caused mild lung and liver phospholipidosis, while the rest (GWC-GWE) did not cause any detectable toxicity. Urine samples were collected predose, 0-8 h, 8-16 h, 16-24 h and 24-32 h after single, oral dosing with each compd. to

Han Wistar rats (n=3 per group), and liver and lung samples for were taken at 48 h for histol. 1H NMR spectra of whole urine were acquired, processed and subsequently analyzed using principal component anal. All animals administered the drug candidates showed a significant redn. in serum triglycerides and those animals administered either GWA or GWB were obsd. to have foamy alveolar macrophages and the presence of multilamellar bodies in hepatocytes by electron microscopy. In the plot of the first two principal components, urinary spectra of those animals dosed with GWA or GWB mapped sep. to controls, all pre-dose samples and animals dosed with GWC-GWE. Inspection of the principal components loadings indicated an increase in urinary phenylacetylglycine with a concomitant decrease in urinary citrate and 2-oxoglutarate, possibly constituting a novel urinary biomarker set for phospholipidosis. This work exemplifies the use of NMR spectroscopy and pattern recognition methods for the detection of novel biomarker combinations for poorly understood toxicity types and the potential in screening novel drugs for toxicity.REFERENCE COUNT: 35

L1 ANSWER 13 OF 38 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2000:772627 CAPLUS
DOCUMENT NUMBER: 133:340314
TITLE: Therapeutic action and properties of a polymorphic form of 5-[4-[2-(N-methyl-N-(2-pyridyl)amino)ethoxy]benzyl]thiazolidine-2,4-dione, maleic acid salt
INVENTOR(S): Blackler, Paul David James; Giles, Robert Gordon; Moore, Stephen; Sasse, Michael John
PATENT ASSIGNEE(S): SmithKline Beecham PLC, UK
SOURCE: PCT Int. Appl., 19 pp.
PATENT NO. KIND DATE APPLICATION NO. DATE
--------------- ---- -------- --------------- --------
WO 2000064893 A2 20001102 WO 2000-GB1522 20000419
WO 2000064893 A3 20010125
EP 1175418 A2 20020130 EP 2000-922793 20000419
BR 2000009935 A 20020416 BR 2000-9935 20000419
JP 2002543076 T2 20021217 JP 2000-614245 20000419
EP 1277753 A1 20030122 EP 2002-80319 20000419
NO 2001005148 A 20011217 NO 2001-5148 20011022
HR 2001000774 A1 20021031 HR 2001-774 20011022
BG 106122 A 20020531 BG 2001-106122 20011120
PRIORITY APPLN. INFO.: GB 1999-9471 A 19990423
GB 1999-12195 A 19990525 EP 2000-922793 A3 20000419
WO 2000-GB1522 W 20000419
AB A polymorphic form of 5-[4-[2-(N-methyl-N-(2-pyridyl)amino)ethoxy]benzyl]t hiazolidine-2,4-dione, maleic acid salt (the "Polymorph") characterized in that it provides: (i) an infra red spectrum contg. peaks at 1752, 1546, 1154, 621, and 602 cm-1; and/or (ii) a Raman spectrum contg. peaks at 1751, 1243 and 602 cm-1; and/or (iii) a solid-state NMR spectrum contg. peaks at 111.9, 114.8, 119.6, 129.2, 134.0, 138.0, 144.7, 153.2, 157.1, 170.7, 172.0 and 175.0 ppm; and/or (iv) an x-ray powder diffraction (XRPD) pattern which gives calcd. lattice spacings of 6.46, 5.39, 4.83, 4.68, 3.71, 3.63,

3.58, and 3.48 Angstroms; a process for prepg. such a compd., a pharmaceutical compn. contg. such a compd. and the use of such a compd. in medicine.

L1 ANSWER 15 OF 38 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2000:574014 CAPLUS
DOCUMENT NUMBER: 133:183156
TITLE: **Process for quality control and standardization of medicinal plant products**
INVENTOR(S): ***Hylands, Peter John; Nicholson, Jeremy Kirk; Holmes, Elaine Claire; Dunn, Michael John***
PATENT ASSIGNEE(S): Oxford Natural Products Plc, UK
SOURCE: PCT Int. Appl., 47 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000047992	A1	20000817	WO 2000-GB428	20000210
EP 1151292	A1	20011107	EP 2000-902767	20000210
BR 2000008122	A	20011113	BR 2000-8122	20000210
GB 2362953	A1	20011205	GB 2001-21376	20000210
JP 2002536664	T2	20021029	JP 2000-598852	20000210
NO 2001003889	A	20011003	NO 2001-3889	20010809

PRIORITY APPLN. INFO.: GB 1999-3011 A 19990210
US 1999-120591P P 19990218 GB 1999-19573 A 19990818
US 1999-149468P P 19990819 GB 1999-28541 A 19991202
US 1999-168382P P 19991202 WO 2000-GB428 W 20000210

AB A process for establishing a std. specification for a medicinal plant material comprises: (i) prepg. a test soln. or test ext. of a sample of the medicinal plant material which is known to possess the or each property required for the std.; (ii) submitting the said soln. or ext. to two or more anal. methods including (a) a combination of NMR spectroscopy and a computer-based pattern recognition technique, and (b) one or more biol. profiling techniques; (iii) obtaining results from the anal. methods used in step (ii); and (iv) establishing a std. specification for the said plant material on the basis of the results obtained in step (iii). Candidate samples of the medicinal plant material may subsequently be tested for compliance with the std. They can be accepted or rejected depending on whether they give anal. results which fall within or outside either part or all of the specification established in step (iv). This approach to standardization and quality control is particularly applicable to mixts. of medicinal plant materials. NMR spectroscopy and multivariate anal. was used to discriminate between sources of Panax ginseng. Ext. of Buddleja globosa was prepd. and its stimulatory effects on human dermal fibrobalst growth was studied.
REFERENCE COUNT: 5

L1 ANSWER 16 OF 38 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2000:559380 CAPLUS
DOCUMENT NUMBER: 134:128019
TITLE: Comparative NMR analysis of stable isotope labeling

patterns. Biosynthesis of gallic acid
AUTHOR(S): Eisenreich, Wolfgang; Werner, Ingo; Bacher, Adelbert
CORPORATE SOURCE: Inst. Organ. Chemie und Biochemie, Technische Universitat Munchen, Garching, D-85747, Germany
SOURCE: NMR in Microbiology (2000), 381-409. Editor(s):Barbotin, Jean-Noel; Portais, Jean-Charles. Horizon Scientific Press: Wymondham, UK.
CODEN: 69ADBD
DOCUMENT TYPE: Conference
LANGUAGE: English
AB The biosynthesis of gallic acid was studied in cultures of the fungus Phycomyces blakesleeanus and in leaves of the tree Rhus typhina by labeling expts. using [1-13C]glucose and a mixt. of [U-13C6]glucose and unlabeled glucose (1:25, wt./wt.). A detailed quant. NMR anal. of gallic acid and amino acids isolated from the cells showed that the bulk amt. of gallic acid is formed directly via 5-dehydroshikimate and not via phenylalanine. The retro-biosynthetic approach used in this study is described in detail and compared with classical approaches of in vivo incorporation expts.REFERENCE COUNT: 26

L1 ANSWER 17 OF 38 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2000:515874 CAPLUS
DOCUMENT NUMBER: 133:190491
TITLE: Solid-state 29Si VACP/MAS NMR studies of silicon-accumulating plants: structural characterization of biosilica deposits
AUTHOR(S): Bertermann, Rudiger; Tacke, Reinhold
CORPORATE SOURCE: Institut fur Anorganische Chemie, Universitat Wurzburg, Wurzburg, D-97074, Germany
SOURCE: Zeitschrift fuer Naturforschung, B: Chemical Sciences (2000), 55(6), 459-461
CODEN: ZNBSEN; ISSN: 0932-0776
PUBLISHER: Verlag der Zeitschrift fuer Naturforschung
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A series of silicon-accumulating plants [different Equisetum (horse tail) species, Echium vulgare, and Symphytum officinale] were studied by solid-state 29Si NMR expts. Selected parts of these plants were freeze-dried and then investigated by solid-state 29Si VACP/MAS NMR spectroscopy. The 29Si NMR spectra of these plants are quite similar and exhibit the typical pattern characteristic of polysilicic acid (amorphous silica).
REFERENCE COUNT: 7

L1 ANSWER 18 OF 38 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2000:128597 CAPLUS
DOCUMENT NUMBER: 132:259995
TITLE: "Accelerated toxicity screening using NMR and pattern recognition-based methods"
AUTHOR(S): *Holmes, Elaine; Shockcor, John P.*

CORPORATE SOURCE: Department of Biological Chemistry, Division of Biomedical Sciences, Imperial College of Science, Technology and Medicine, London, SW7 2AZ, UK
SOURCE: **Current Opinion in Drug Discovery & Development (2000), 3(1), 72-78**
CODEN: CODDFF; ISSN: 1367-6733
PUBLISHER: PharmaPress Ltd.
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
AB A review with 33 refs. 1H-NMR spectroscopy has proved to be a powerful and efficient means of monitoring the interaction of pharmacol. agents with cells and tissues [1.bul.]. The application of this technique to biofluid anal., gives rise to a comprehensive metabolic profile of the low mol. wt. components of biofluids, that reflect concns. and fluxes of endogenous metabolites involved in key intermediary cellular pathways, thereby giving an indication of an organism's physiol. or pathophysiol. status [1.bul.]. Recent developments in spectrometer technol. have resulted in increased sensitivity and dispersion. Together with the increased capacity for sample throughput (.apprx. 300 samples/day), arising from the latest advances in flow probe technol. and in robotic transfer systems [2], 1H-NMR spectroscopic techniques have become viable in terms of toxicol. screening. However, the complexity of high-field biofluid spectra in conjunction with the increased capacity for sample handling, leading to a rapid growth in the size of toxicol. spectral databases, has placed greater emphasis on the need to develop improved automated procedures for data processing and interpretation. By harnessing chemometric tools to the anal. of complex spectral data, the toxicol. consequences of xenobiotic exposure can be evaluated efficiently online. Automation of spectral processing procedures and the construction of math.-based "expert systems" for the prediction of drug-induced toxicity founded on 1H-NMR spectral profiles, have now been achieved. In this article, we review the recent developments in NMR and pattern recognition anal. and consider their application in toxicol. screening.
REFERENCE COUNT: 33

L1 ANSWER 19 OF 38 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1999:769517 CAPLUS
DOCUMENT NUMBER: 132:58602
TITLE: "Metabonomics": understanding the metabolic responses of living systems to pathophysiological stimuli via multivariate statistical analysis of biological NMR spectroscopic data"
AUTHOR(S): *Nicholson, J. K.; Lindon, J. C.; Holmes, E.*
CORPORATE SOURCE: Biological Chemistry, Biomedical Sciences Division, Imperial College of Science, Technology and Medicine, University of London, London, SW7 2AZ, UK
SOURCE: **Xenobiotica (1999), 29(11), 1181-1189**
CODEN: XENOBH; ISSN: 0049-8254
PUBLISHER: Taylor & Francis Ltd.
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English

AB A review with many refs. on properties of metabonomic data sets and the possible uses of NMR-based metabonomics for toxicol. classification and biomarker or surrogate marker identification in vivo.
REFERENCE COUNT: 28

L1 ANSWER 20 OF 38 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1998:720944 CAPLUS
DOCUMENT NUMBER: 130:233339
TITLE: "The identification of novel biomarkers of renal toxicity using automatic data reduction techniques and PCA of proton NMR spectra of urine"
AUTHOR(S): *Holmes, Elaine; Nicholson, Jeremy K.; Nicholls, Andrew W.; Lindon, John C.; Connor, Susan C.; Polley, Stephen; Connelly, John*
CORPORATE SOURCE: Birkbeck College, Department of Chemistry, University of London, London, SW7 2AZ, UK
SOURCE: **Chemometrics and Intelligent Laboratory Systems (1998), 44(1,2), 245-255**
CODEN: CILSEN; ISSN: 0169-7439
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Early detection of drug-induced toxic lesions is of considerable importance in the pharmaceutical industry. Many drugs and toxins produce characteristic patterns of biochem. perturbations in the urinary profile related to the site or mechanism of the lesion. NMR spectroscopy of biofluids has been shown to be a useful technique for characterizing such lesions. We present here an efficient approach to the anal. and classification of complex urine NMR spectra obtained from rats treated with various nephrotoxins (glomerular, papillary and proximal tubular) based on the automatic generation of descriptors for the spectra with subsequent PCA. Urinalysis was performed using 600 MHz NMR spectroscopy and the site of renal lesion was confirmed by renal histol. A plot of the first three PCs showed distinct clustering of urine samples reflecting the site of toxicity within the kidney. Interrogation of the eigenvectors showed which NMR spectral regions contributed most to the sepn. of classes. These regions were examd. visually for perturbations in metabolite profile and sets of 'marker' metabolites that characterized tissue-specific lesions were defined. These studies have shown that automatic data redn. of the spectra followed by multivariate techniques such as principal components anal. (PCA) is a reliable method for screening for biomarkers of organ or tissue-specific chem.-induced lesions.
REFERENCE COUNT: 13

L1 ANSWER 22 OF 38 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1997:785434 CAPLUS
DOCUMENT NUMBER: 128:43365
TITLE: "Flow injection proton nuclear magnetic resonance spectroscopy combined with pattern recognition methods: implications for rapid structural studies and high throughput biochemical screening"

AUTHOR(S): *Spraul, Manfred; Hofmann, Martin; Ackermann, Michael; Nicholls, Andrew W.; Damment, Stephen, J. P.; Haselden, John N.; Shockcor, John P.; Nicholson, Jeremy K.; Lindon, John C.*
CORPORATE SOURCE: Bruker Analytische Messtechnik GmbH, Rheinstetten, D-76287, Germany
SOURCE: **Analyst (Cambridge, United Kingdom) (1997), 122(11), 339-341**
CODEN: ANALAO; ISSN: 0003-2654
PUBLISHER: Royal Society of Chemistry
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The applicability of novel NMR flow probe technol. has been tested by the measurement of 300 MHz 1H NMR spectra of a series of rat urine samples. Compared with conventional automatic operation, the method resulted in a significantly increased rate of sample throughput, required minimal spectrometer optimization before each measurement and avoided the needed for expensive and fragile NMR sample tubes. The NMR approach has been coupled with computer methods for spectral data redn. and classification using, in this case, principal components anal. The flow probe NMR approach offers distinct advantages in situations where large nos. of samples require NMR anal. in a short period of time. These could include routine samples from high throughput chem. synthesis, biofluid samples for drug toxicity monitoring as shown here, samples for clin. diagnosis or real-time anal. in chem. prodn. facilities.

L1 ANSWER 23 OF 38 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1997:3670 CAPLUS
DOCUMENT NUMBER: 126:59027
TITLE: "Multivariate statistical analysis of two-dimensional NMR data to differentiate grapevine cultivars and clones"
AUTHOR(S): *Vorveille, Laurence; Vercauteren, Joseph; Rutledge, Douglas N.*
CORPORATE SOURCE: Lab. Pharmacognosie, Univ. Bordeaux II, Bordeaux, 33076, Fr.
SOURCE: **Food Chemistry (1996), 57(3), 441-450**
CODEN: FOCHDJ; ISSN: 0308-8146
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Multivariate statistical methods were applied on two-dimensional NMR data (1H-13C) of polyphenol exts. from grapevine clones harvested in the Bordeaux region. An anal. of variance detected the most discriminating NMR spectral correlation vols. which were then used to perform principal component, hierarchical clustering and discriminant analyses. The results showed that clones are divided into three groups according to the cultivar and they can be differentiated inside each cultivar. This method was applied to polyphenol exts. from grape seeds and leaves. Two-dimensional NMR is the only anal. tool which can differentiate grapevine clones. It could thus be used to check the identity of grapevine species, cultivars or clones as well as any other plants which produce polyphenols.

L1 ANSWER 24 OF 38 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1995:987472 CAPLUS
DOCUMENT NUMBER: 124:49895
TITLE: "Plant histochemistry by correlation peak imaging"
AUTHOR(S): *Metzler, A.; Izquierdo, M.; Ziegler, A.; Koeckenberger, W.; Komor, E.; von Kienlin, M.; Haase, A.; Decorps, M.*
CORPORATE SOURCE: Physikalisches Inst. V, Univ. Wuerzburg, Wuerzburg, 97074, Germany
SOURCE: **Proceedings of the National Academy of Sciences of the United States of America (1995), 92(25), 11912-15**
CODEN: PNASA6; ISSN: 0027-8424
PUBLISHER: National Academy of Sciences
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Using a new NMR correlation-peak imaging technique, the authors were able to investigate noninvasively the spatial distribution of carbohydrates and amino acids in the hypocotyl of castor bean seedlings. In addn. to the expected high sucrose concn. in the phloem area of the vascular bundles, the authors could also observe high levels of sucrose in the cortex parenchyma, but low levels in the pith parenchyma. In contrast, the glucose concn. was lower in the cortex parenchyma than in the pith parenchyma. Glutamine and/or glutamate was detected in the cortex parenchyma and in the vascular bundles. Lysine and arginine were mainly visible in the vascular bundles, whereas valine was obsd. in the cortex parenchyma, but not in the vascular bundles. Although the physiol. significance of these metabolite distribution patterns is not known, they demonstrate the potential of spectroscopic NMR imaging to study noninvasively the physiol. and spatial metabolic heterogeneity of living plants.

L1 ANSWER 25 OF 38 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1995:875341 CAPLUS
DOCUMENT NUMBER: 123:280086
TITLE: "Using multivariate methods on solid-state 13C NMR data of complex materials"
AUTHOR(S): *Karlstroem, Hans; Nilsson, Mats; Norden, Bo*
CORPORATE SOURCE: Kimit AB, Kiruna, S-981 86, Swed.
SOURCE: **Analytica Chimica Acta (1995), 315(1-2), 1-14**
CODEN: ACACAM; ISSN: 0003-2670
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Multivariate data anal. (MVA) has been used as an aid in the anal. and interpretation of 13C NMR spectra in the solid state. The goal of this study was to investigate the effect of some important instrumental parameters and calcn. strategies on the outcome of the multivariate data anal. The samples used were two peat forming plants, Sphagnum fuscum and Carex rostrata, incubated in four different redox environments. It was found that normalizing each NMR spectrum to a const. area should be avoided. Using non-normalized data we get a slightly better class sepn. and the peaks in the 'subspectra' are sharpened. Depending on the relative size of interesting variation one should be careful

when choosing the no. of variables, i.e. no. of data points characterizing each spectrum. The line broadening technique should be used with great care in order not to obscure the information. We also suggest the use of the free induction decay (FID)/MVA directly for classification purposes. This is a new approach to analyze the output data from NMR measurements.

L1 ANSWER 26 OF 38 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1995:779836 CAPLUS
TITLE: Finger-like lysing patterns of blood clots
AUTHOR(S): Zidansek, Aleksander; Blinc, Ales; Lahajnar, Gojmir; Keber, Dusan; Blinc, Robert
CORPORATE SOURCE: J. Stefan Inst., Univ. of Ljubljana, Ljubljana, 61000, Slovenia
SOURCE: Biophysical Journal (1995), 69(3), 803-9
CODEN: BIOJAU; ISSN: 0006-3495
PUBLISHER: Biophysical Society
DOCUMENT TYPE: Journal
LANGUAGE: English
AB One-dimensional modeling of fibrinolysis (Senf, 1979; Zidansek and Blinc, 1991; Diamond and Anand, 1993) has accounted for the dissoln. velocity, but the shape of the lysing patterns can be explained only by two- or three-dimensional models. Here we report on finger-like drug -induced blood clot dissoln. patterns obtained by proton NMR imaging, which can be described by the enzyme transport-limited system of fibrinolytic chem. equations with diffusion and perfusion terms (Zidansek and Blinc, 1991) in the reaction time approxn. if the random character of gel porosity is taken into account. A two-dimensional calcn. based on the hele-Shaw random walk models (Kadanoff, 1985; Liang, 1986) leads to fractal lysing patterns as, indeed, is obsd. The fractal dimension of the exptl. lysing patterns changes from 1.2 at the beginning of the expts. to a max. of .apprx.1.3 in the middle and then decreases toward one when the clot is recanalized.

L1 ANSWER 27 OF 38 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1995:484723 CAPLUS
DOCUMENT NUMBER: 122:255333
TITLE: Proton NMR observation of the antineoplastic agent Iproplatin in vivo by selective multiple quantum coherence transfer (Sel-MQC)
AUTHOR(S): He, Qiuhong; Bhujwalla, Zaver M.; Maxwell, Ross J.; Griffiths, John R.; Glickson, Jerry D.
CORPORATE SOURCE: School of Medicine, Johns Hopkins University, Baltimore, MD, 21025-2195, USA
SOURCE: Magnetic Resonance in Medicine (1995), 33(3), 414-16
CODEN: MRMEEN; ISSN: 0740-3194
DOCUMENT TYPE: Journal
LANGUAGE: English
AB We have noninvasively detected the proton signal of an antineoplastic agent Iproplatin in vivo by selective multiple quantum coherence transfer (Sel-MQC). Without isotopic labeling or chem. modification, the Sel-MQC method labels Iproplatin by its intrinsic proton multiple quantum coherences and, hence, differentiates the Iproplatin

signal from the intensive overlapping resonances of lipid and lactate. This proton NMR method should also be applicable to study other drugs with appropriate spin coupling patterns.

L1 ANSWER 28 OF 38 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1994:504200 CAPLUS
DOCUMENT NUMBER: 121:104200
TITLE: Stereochemistry of quinolizidine alkaloid biosynthesis: incorporation of the enantiomeric [2-2H]cadaverines into lupinine
AUTHOR(S): Robins, David J.; Sheldrake, Gary N.
CORPORATE SOURCE: Department of Chemistry, University of Glasgow, Glasgow, G12 80Q, UK
SOURCE: Journal of the Chemical Society, Chemical Communications (1994), (11), 1331-2
CODEN: JCCCAT; ISSN: 0022-4936
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Samples of (R)- and (S)-[2-2H]cadaverines prepd. from L- and D-glutamic acid, resp., were fed to Lupinus luteus plants and the labeling patterns in lupinine detd. by 2H NMR spectroscopy demonstrated that the quinolizidine ring system is formed by removal of the pro-S hydrogen and retention of the pro-R hydrogen at C-1 of lupinine.

L1 ANSWER 29 OF 38 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1991:553327 CAPLUS
DOCUMENT NUMBER: 115:153327
TITLE: Proton NMR spectra of vertebrate iron-sulfur [2Fe-2S] ferredoxins. Hyperfine resonances suggest different electron delocalization patterns from plant ferredoxins
AUTHOR(S): Skjeldal, Lars; Markley, John L.; Coghlan, Vincent M.; Vickery, Larry E.
CORPORATE SOURCE: Coll. Agric. Life Sci., Univ. Wisconsin, Madison, WI, 53706, USA
SOURCE: Biochemistry (1991), 30(37), 9078-83
CODEN: BICHAW; ISSN: 0006-2960
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The observation of paramagnetically shifted (hyperfine) proton resonances from vertebrate mitochondrial [2Fe-2S] ferredoxins is reported. The hyperfine signals of human, bovine, and chick [2Fe-2S] ferredoxins are described and compared with those of Anabena 7120 vegetative ferredoxin, a plant-type [2Fe-2S] ferredoxin studied previously. The hyperfine resonances of the three vertebrate ferredoxins were very similar to one another both in the oxidized state and in the reduced state, and slow (on the NMR scale) electron self-exchange was obsd. in partially reduced samples. For the oxidized vertebrate ferredoxins, hyperfine signals were obsd. downfield of the diamagnetic envelope from +13 to +50 ppm, and the general pattern of peaks and their anti-Curie temp. dependence are similar to those obsd. for the oxidized plant-type ferredoxins. For the reduced vertebrate ferredoxins, hyperfine signals were obsd. both upfield (-2 to -18 ppm) and downfield (+15 to +45 ppm), and all were found to exhibit Curie-type temp.

dependence. This pattern and temp. dependence are distinctly different from those previously found with reduced plant-type ferredoxins which have signals centered around +120 ppm with Curie-type temp. dependence, assigned to cysteines which interact with Fe(III), and signals centered around +20 ppm with anti-Curie temp. dependence, assigned to cysteines which interact with Fe(II). The contact-shifted resonances in the reduced vertebrate ferredoxins detect different spin magnetization from those in the reduced plant ferredoxins and it is suggested that plant and vertebrate ferredoxins have fundamentally different patterns of electron delocalization in the reduced [2Fe-2S] center.

L1 ANSWER 30 OF 38 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1991:156545 CAPLUS
DOCUMENT NUMBER: 114:156545
TITLE: Tracing the human metabolism of stable isotope-labeled drugs by ex vivo NMR spectroscopy. A revision of S-carboxymethyl-L-cysteine biotransformation
AUTHOR(S): Meese, Claus O.; Fischer, Peter
CORPORATE SOURCE: Dr. Margarete Fischer-Bosch-Inst. Klin. Pharmacol., Stuttgart, D-7000/50, Germany
SOURCE: Zeitschrift fuer Naturforschung, C: Journal of Biosciences (1990), 45(11-12), 1171-5
CODEN: ZNCBDA; ISSN: 0341-0382
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A direct structural identification, and quant. assessment below the 50 nmol/mL level, of the full pattern of renally excreted metabolites is made possible by 13C-NMR measurements of untreated urine samples when stable isotope-labeled (13C) drug analogs are administered to humans. The full potential of the new ex vivo NMR approach is exemplified by a study, for a group of volunteers, of S-carboxymethyl-L-cysteine metab. The metabolic sulfoxidn. pathway of S-carboxymethyl-L-cysteine in man, accepted so far, needs to be profoundly revised on the basis of the 13C-NMR results.

L1 ANSWER 31 OF 38 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1990:584279 CAPLUS
DOCUMENT NUMBER: 113:184279
TITLE: Structure and dynamics of distamycin A with d(CGCAAATTGGC):d(GCCAATTTGCG) at low drug:DNA ratios
AUTHOR(S): Pelton, Jeffrey G.; Wemmer, David E.
CORPORATE SOURCE: Lawrence Berkeley Lab., Univ. California, Berkeley,CA, 94720, USA
SOURCE: Journal of Biomolecular Structure & Dynamics (1990), 8(1), 81-97
CODEN: JBSDD6; ISSN: 0739-1102
DOCUMENT TYPE: Journal
LANGUAGE: English
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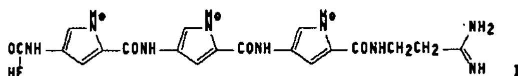

AB Two-dimensional NMR has been used to study the interaction of distamycin A (I) with d(CGCAAATTGGC):d(GCCAATTTGCG) at low and intermediate drug:DNA ratios (<2.0). Drug-DNA contacts were identified by nuclear Overhauser effect spectroscopy (NOEs), which also served to monitor exchange of the drug between different binding sites. At low drug:DNA ratios (0.5), I binds in two orientations within the five central A-T base pairs and has a preference (2.2:1) for binding with the formyl end directed toward the 5' side of the A-rich strand. The pattern of drug-DNA contacts corresponding to the preferred binding orientation are consistent with the drug sliding between adjacent AAAT and AATT binding sites at a rate that is fast on the NMR time scale. Similarly, the pattern of NOEs assocd. with the less favored orientation are consistent with the drug sliding between adjacent AATT and ATTT sites, again in fast exchange. Off-rates for the drug from the major and minor binding orientations were measured to be 2.4 and 3.3 s-1, resp., at 35.degree.. At intermediate drug:DNA ratios (1.3), exchange of the drug between the two one-drug and the two sites of a two-drug complex is obsd. Off-rates for both drugs from the 2:1 complex were measured to be 1.0 s-1 (35.degree.).

L1 ANSWER 32 OF 38 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1990:512729 CAPLUS
DOCUMENT NUMBER: 113:112729
TITLE: GC-MS and 13C-NMR studies on the biosynthesis of terpenoid defensive secretions by the larvae of papilionid butterflies (Luehdorfia and Papilio)
AUTHOR(S): Honda, Keiichi
CORPORATE SOURCE: Seisho Biol. Lab., Odawara, 250, Japan
SOURCE: Insect Biochemistry (1990), 20(3), 245-50
CODEN: ISBCAN; ISSN: 0020-1790
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The biosynthetic pathway of some terpenic hydrocarbons present in the larval osmeterial secretions of Luehdrofa (homogeneous type) and Papilio (heterogeneous type) species were examd. by in vivo expts., using [13C]acetic acid which was topically applied to the everted osmeteria. Gas chromatog./mass spectrometry (GC-MS) investigation demonstrated that 13C was incorporated into mono- and(or) sesquiterpene hydrocarbons with an enrichment factor of .apprx.0.5% (L. puziloi), 1.0% (P. protenor), and 2.9% (P. helenus) by treatment with [1,2-13C]acetic acid, thereby substantiating de novo biosynthesis of terpenes from acetate precursors by these larvae. The incorporation pattern of [2-13C]- or [1,2-13C]acetic acid into the C framework of .beta.-myrcene (L. puziloi) and (E)-.beta.-farnesene (P. helenus) as revealed by 13C-NMR spectroscopy definitely elucidated the biosynthesis of terpenic compds. in both species by the familiar terpenoid synthetic system with the isoprenoid skeletal units that is widely known in plants. Partial correction of previous assignment of 13C-NMR spectra of .beta.-myrcene and (E)-.beta.-farnesene is also made.

L1 ANSWER 33 OF 38 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1990:157940 CAPLUS
DOCUMENT NUMBER: 112:157940

TITLE: Photo-CIDNP NMR studies of drugs of the central nervous system derived from indole and phenol rings
AUTHOR(S): Consonni, Roberto; De Marco, Antonio; Zannoni, Giulio; Zetta, Lucia; Dijkstra, Klaas
CORPORATE SOURCE: Ist. Chim. Macromol., CNR, Milan, I-20131, Italy
SOURCE: Gazzetta Chimica Italiana (1989), 119(9), 475-80
CODEN: GCITA9; ISSN: 0016-5603
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Photochem.-induced dynamic nuclear polarization combined with 1H NMR have been used to investigate the structure of a no. of drugs sharing a common moiety. From the photo-CIDNP NMR spectra of central nervous system drugs such as morphine, adrenaline, serotonin and lysergic acid, a common pattern emerges for the polarizability of protons in the arom. region of the drugs, or in its neighborhood. The photo-CIDNP technique, used so far mainly for the investigation of arom. residues in proteins, is offered as a novel approach for structural studies of small mols. of biol. or pharmacol. interest. The high sensitivity of the technique makes it also quite promising for studies of substances of very low soly., or available only in very small amts., as is often the case for metabolites.

L1 ANSWER 34 OF 38 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1990:18339 CAPLUS
DOCUMENT NUMBER: 112:18339
TITLE: High resolution proton magnetic resonance spectroscopy of biological fluids
AUTHOR(S): Nicholson, Jeremy K.; Wilson, Ian D.
CORPORATE SOURCE: Birkbeck Coll., Univ. London, London, SC1H 0PP, UK
SOURCE: Progress in Nuclear Magnetic Resonance Spectroscopy (1989), 21(4-5), 449-501
CODEN: PNMRAT; ISSN: 0079-6565
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
AB A review, with 129 refs., the title subject with emphasis on practical considerations of 1H-NMR of biofluids; the biochem., physicochem., and NMR properties of humans and animal body fluids; clin. applications; application in drug metab.; toxicol. applications; and pattern recognition approaches to the interpretation of NMR generated toxicol. data.

L1 ANSWER 35 OF 38 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1988:507787 CAPLUS
DOCUMENT NUMBER: 109:107787
TITLE: Regulation of malic acid metabolism in Crassulacean acid metabolism plants in the dark and light: in vivo evidence from 13C-labeling patterns after 13C-carbon dioxide fixation
AUTHOR(S): Osmond, C. B.; Holtum, J. A. M.; O'Leary, M. H.; Roeske, C.; Wong, O. C.; Summons, R. E.; Avadhani, P. N.

CORPORATE SOURCE: Res. Sch. Biol. Sci., Aust. Natl. Univ., Canberra, 2601, Australia
SOURCE: Planta (1988), 175(2), 184-92
CODEN: PLANAB; ISSN: 0032-0935
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The labeling patterns in malic acid from dark 13CO2 fixation in 7 species of succulent plants with Crassulacean acid metab. were analyzed by gas chromatog.-mass spectrometry and 13C-NMR spectrometry. Only singly labeled malic acid mols. were detected and on the av., after 12-14 h dark 13CO2 fixation the ratio of [4-13C] to [1-13C] label was 2:1. However, the 4-C carboxyl contained from 72 to 50% of the label depending on species and temp. The 13C enrichment to malate and fumarate was similar. These data indicate that fumarase randomization is responsible for movement of label to 1-C malic acid following carboxylation of phosphoenolpyruvate. The extent of randomization may depend on time and on the balance of malic acid fluxes between mitochondria and vacuoles. The ratio of labeling in 4-C to 1-C of malic acid which accumulated following 13CO2 fixation in the dark did not change during deacidification in the light and no doubly-labeled mols. of malic acid were detected. Also, fumarase randomization does not occur in the light, and futile cycling of decarboxylation products of [13C]malic acid (13CO2 or [1-13C]pyruvate) through phosphoenolpyruvate carboxylase does not occur, presumably because malic acid inhibits this enzyme in the light in vivo. Short-term exposure to 13CO2 in the light after deacidification leads to the synthesis of singly and multiply labeled malic acid in these species. In the shortest times, only singly-labeled [4-13C]malate was detected but this may be a consequence of the higher intensity and better detection statistics of this ion cluster during mass spectrometry. Thus, both phosphoenolpyruvate carboxylase (EC 4.1.1.32) and ribulose-1,5-bisphosphate carboxylase (EC 4.1.1.39) are active at this time.

L1 ANSWER 36 OF 38 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1987:420792 CAPLUS
DOCUMENT NUMBER: 107:20792
TITLE: Pyrrolizidine alkaloid biosynthesis. Incorporation of carbon-13-labeled precursors into rosmarinine
AUTHOR(S): Kelly, Henry A.; Robins, David J.
CORPORATE SOURCE: Dep. Chem., Univ. Glasgow, Glasgow, G12 8QQ, UK
SOURCE: Journal of the Chemical Society, Perkin Transactions 1: Organic and Bio-Organic Chemistry (1972-1999) (1987), (1), 177-80
CODEN: JCPRB4; ISSN: 0300-922X
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Biosynthesis of the rosmarinecine portion of the pyrrolizidine alkaloid rosmarinine was investigated in Senecio pleistocephalus using 13C-labeled precursors. Plants were fed with [1-13C]putrescine dihydrochloride and [2,3-13C2]putrescine dihydrochloride, and the labeling patterns in the biosynthetically derived rosmarinine were established by 13C NMR spectroscopy. Two mols. of putrescine were incorporated to about the same extent into rosmarinecine. Incorporation of [1-amino-15N,1-13C]putrescine

dihydrochloride into rosmarinine produced a labeling pattern which was consistent with conversion of the 2 putrescine mols. into a C4-N-C4 sym. intermediate. This intermediate was identified as homospermidine by feeding [1,9-13C2]homospermidine trihydrochloride to S. pleistocephalus plants. Intact incorporation of this precursor was demonstrated by observation of 2 enriched 13C NMR signals for C-8 and C-9 of rosmarinine.

L1 ANSWER 37 OF 38 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1984:118008 CAPLUS
DOCUMENT NUMBER: 100:118008
TITLE: Some plant leaves have orientation-dependent EPR and NMR spectra
AUTHOR(S): McCain, Douglas C.; Selig, Ted C.; Govindjee; Markley, John L.
CORPORATE SOURCE: Dep. Chem., Univ. South. Mississippi, Hattiesburg, MS, 39401, USA
SOURCE: Proceedings of the National Academy of Sciences of the United States of America (1984), 81(3), 748-52
CODEN: PNASA6; ISSN: 0027-8424
DOCUMENT TYPE: Journal
LANGUAGE: English
AB 1H-NMR spectra of leaves from 50 plants species were obtained at a spectrometer frequency of 470 MHz. Water present in leaf samples gave rise to characteristic spectral patterns. Most species showed only one broad 1H-NMR peak; however, the leaves of some plants displayed complex, orientation-dependent spectra in which a common 3-line pattern was discerned. The pattern varied with the angle between the leaf surface and the external magnetic field. Proton relaxation measurements showed the presence of .gtoreq.2 water compartments in the leaves. The compartments were responsible for different components of the spectral pattern. EPR spectra, obtained at 35 GHz and at -180.degree., of plant leaf sections were dominated by the strong signals of manganous ions. Most plant leaves exhibited isotropic Mn2+ EPR spectra. However, in some species (including ones that exhibit orientation-dependent 1H-NMR spectra) orientation-dependent intensities in the forbidden lines were detected; the spectra indicate that Mn2+ occupied binding sites with axial or lower symmetry on nonrandomly oriented membranes. Both the NMR and the EPR results suggest that the chloroplasts of some plants are preferentially aligned with respect to the leaf surface.

L1 ANSWER 38 OF 38 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1971:30578 CAPLUS
DOCUMENT NUMBER: 74:30578
TITLE: Effect of alkyl substitution in drugs. XXIII. Effect of alkyl substitution on the activity pattern of diphenhydramine. IR and NMR spectral data
AUTHOR(S): Rekker, Roelof F.; Nauta, Wijbe T.
CORPORATE SOURCE: Res. Dep., N. V. K. Pharm. Fabr. v/h Brocades-Stheeman Pharm., Amsterdam, Neth.
SOURCE: Arzneimittel-Forschung (1970), 20(10), 1572-4
CODEN: ARZNAD; ISSN: 0004-4172
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The ir spectra of diphenhydramine derivs. (substituted benzhydrols and their 2-dimethylaminoethyl ethers) were measured in the region of out-of-plane bending vibrations of the C-H aromatic bonds (700-850 cm-1). The effects of structural variations on the chem. shift of the central H atoms of the benzhydryl part were also examd. in the NMR spectrum. In benzhydrols and their ethers the central H was distinctly less acidic than expected. P-Methyl substitution further decreased acidity, whereas o-methyl substitution distinctly increased it. The chem. shift of the central H strongly depended on the electronegativity of the alc. or ether O, and this was detd. by the extent to which the orbitals of the free O electrons participated in overlap with the .pi.-electron system. Attempts to correlate the biol. data of the 2-dimethylaminoethyl ethers with the NMR shift values showed that high antihistaminic activity is coupled with low shift value, whereas high anticholinergic activity is accompanied by a high shift value.

Cancer Chemother Pharmacol (2003) 52 (Suppl 1): S45–S56
DOI 10.1007/s00280-003-0593-0

Paul Workman

The opportunities and challenges of personalized genome-based molecular therapies for cancer: targets, technologies, and molecular chaperones

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Abstract There are now unprecedented opportunities for the development of improved drugs for cancer treatment. Following on from the Human Genome Project, the Cancer Genome Project and related activities will define most of the genes in the majority of common human cancers over the next 5 years. This will provide the opportunity to develop a range of drugs targeted to the precise molecular abnormalities that drive various human cancers and opens up the possibility of personalized therapies targeted to the molecular pathology and genomics of individual patients and their malignancies. The new molecular therapies should be more effective and have less-severe side effects than cytotoxic agents. To develop the new generation of molecular cancer therapeutics as rapidly as possible, it is essential to harness the power of a range of new technologies. These include: genomic and proteomic methodologies (particularly gene expression microarrays); robotic high-throughput screening of diverse compound collections, together with *in silico* and fragment-based screening techniques; new structural biology methods for rational drug design (especially high-throughput X-ray crystallography and nuclear magnetic resonance); and advanced chemical technologies, including combinatorial and parallel synthesis. Two major challenges to cancer drug discovery are: (1) the ability to convert potent and selective lead compounds with activity by the desired mechanism on tumor cells in culture into agents with robust, drug-like properties, particularly in terms of pharmacokinetic and metabolic properties; and (2) the development of validated pharmacodynamic endpoints and molecular markers of drug response, ideally using noninvasive imaging technologies. The use of various new technologies will be exemplified. A major conceptual and practical issue facing the development and use of the new molecular cancer therapeutics is whether a single drug that targets one of a series of key molecular abnormalities in a particular cancer (e.g. BRAF) will be sufficient on its own to deliver clinical benefit ("house of cards" and tumor addiction models). The alternative scenario is that it will require either a combination of agents or a class of drug that has downstream effects on a range of oncogenic targets. Inhibitors of the heat-shock protein (HSP) 90 molecular chaperone are of particular interest in the latter regard, because they offer the potential of inhibiting multiple oncogenic pathways and simultaneous blockade of all six "hallmark traits" of cancer through direct interaction with a single molecular drug target. The first-in-class HSP90 inhibitor 17AAG exhibited good activity in animal models and is now showing evidence of molecular and clinical activity in ongoing clinical trials. Novel HSP90 inhibitors are also being sought. The development of HSP90 inhibitors is used to exemplify the application of new technologies in drug discovery against a novel molecular target, and in particular the need for innovative pharmacodynamic endpoints is emphasized as an essential component of hypothesis-testing clinical trials.

Keywords Molecular pathology and genomics of cancer · New molecular targets · Technologies for drug discovery and development · HSP90 molecular chaperone inhibitors · Gefitinib · Imatinib · Trastuzumab

This work was presented at the 18th Bristol-Myers Squibb Nagoya International Cancer Treatment Symposium, "New Strategies for Novel Anticancer Drug Development," 8–9 November 2002, Nagoya, Japan

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Introduction

In many ways cancer drug discovery is unrecognizable from what it was even as little as 10 years ago. The

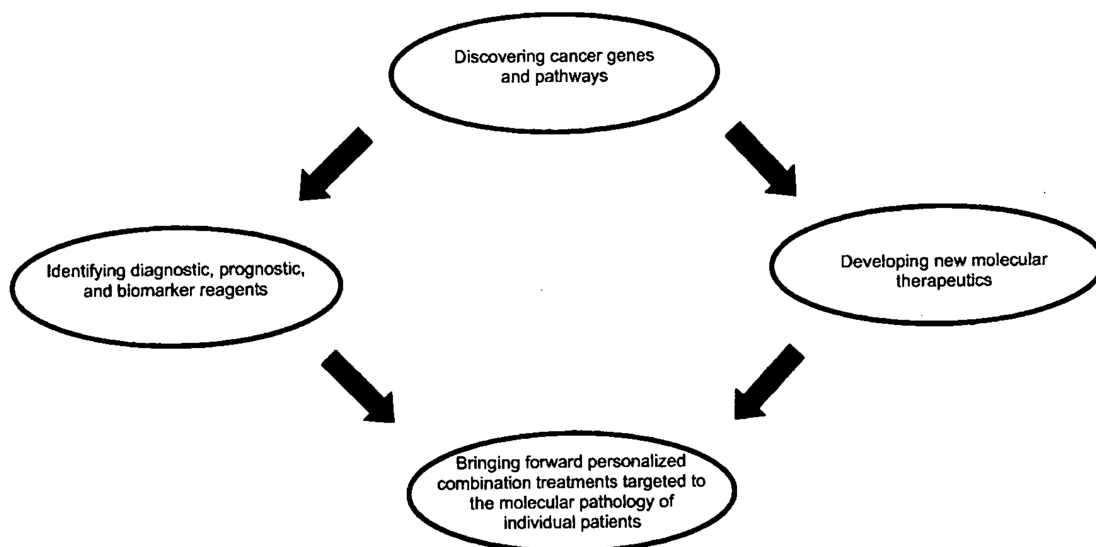
progressive elucidation of the molecular control pathways that are hijacked by cancers has provided us with a large number of potential targets for therapeutic intervention. At the same time, the putting together of a powerful tool kit of innovative technologies has allowed us to accelerate the pace and improve the efficiency of drug discovery [44].

Hence the focus of the first part of this commentary is on new targets and technologies. To illustrate how new drug discovery and development is now done, the second part of the article comprises a summary and update on the development of inhibitors of the heat-shock protein (HSP) 90 molecular chaperone. These are of particular interest because they provide a potential approach to block combinatorial oncogenesis within a single drug molecule. In addition, the first-in-class HSP90 inhibitor 17AAG is just completing phase I trials with promising early results.

From cancer genes to individualized therapies

Given that we now understand in increasing detail the molecular abnormalities that drive the process of malignant progression, the major strategy for drug discovery in cancer is to identify the genes and cognate biochemical pathways that are hijacked in cancer cells, to discover molecular reagents and biomarkers to identify pathways with these defects, and to develop drugs that counteract or exploit the deregulated control mechanisms. The vision is that we can exploit our growing knowledge of cancer genes and pathways by developing personalized therapies targeted to the molecular pathology of individual patients and their malignancies (see references 44 and 49, and Fig. 1).

Fig. 1 Strategy for exploiting knowledge of cancer genes and pathways in the development of personalized therapies targeted to molecular pathology of individual patients



A range of drugs that target the molecular pathology of cancer are now undergoing clinical trial (e.g. see reference 49, and Table 1). Proof of concept for the approach is provided by the regulatory approval of imatinib (Gleevec), trastuzumab (Herceptin), and gefitinib (Iressa). Various small-molecule cyclin-dependent kinase inhibitors, e.g. flavopiridol and CYC202 (*R*-roscovitine), are undergoing clinical evaluation. Furthermore, a wide range of innovative agents are in preclinical and clinical development. These include drugs that block the farnesylation of RAS and other protein targets; inhibitors of signal transduction kinases such as RAF-1, MEK, mTOR, and PI3 kinase; and drugs that block chromatin remodeling enzymes such as histone deacetylases [49].

The success with the first initial wave of molecular therapeutics that specifically attack the oncogenic pathways that are hijacked by cancer genome defects has provided encouragement for the view that this represents a major opportunity to develop innovative cancer drugs. Furthermore, the mechanism of action of these agents offers potential not only for improved therapeutic efficacy, but also for less-severe side effects compared with the previous generation of cytotoxic agents. The new agents may in fact be much more like tamoxifen—used chronically for long-term disease control and potentially for chemoprevention.

Additional new targets from cancer genomics

A further tranche of new targets and drugs can be expected to emerge over the next 5–10 years as the genes involved in all stages of the malignant progression of every tumor type are elucidated. Historically, cancer genes have been discovered and cloned by a variety of means, including the dissection of major chromosomal abnormalities, i.e. translocations, amplifications, and deletions; transfection of dominant oncogenes into

Table 1 Examples of novel drugs acting on cancer genome targets (for further details see reference 49)

Imatinib	A small molecule that shows activity in chronic myeloid leukemia and gastrointestinal stromal tumors via inhibition of the BCR-ABL and c-KIT receptor tyrosine kinases, respectively
Trastuzumab	A monoclonal antibody active in ERBB2-positive breast cancers
Gefitinib	A small-molecule inhibitor of the epidermal growth factor receptor tyrosine kinase active in non-small-cell lung, hormone-refractory prostate, and head and neck cancer
Various small-molecule cyclin-dependent kinase inhibitors, e.g. flavopiridol and CYC202 (*R*-roscovitine)	Undergoing clinical evaluation
Inhibitors of RAS farnesylation, RAF-1, MEK, PI3 kinase, mTOR, and histone deacetylases	In preclinical and clinical development
Wide range of other innovative agents	In preclinical and clinical development, e.g. potential for BRAF inhibitors
17AAG	A small-molecule inhibitor of the HSP90 molecular chaperone that is completing phase I clinical with promising early results

NIH3T3 cells; various genetic and molecular studies in model organisms such as yeast, fly, and worm; and also from studies of inherited predisposition [31].

The discovery of new cancer genes should be accelerated by the impact of the Cancer Genome Project [42]. The aim here is to use the information and technologies obtained via the Human Genome Project [18, 38] to carry out a systematic, high-throughput, genome-wide screen for somatic mutations in human cancer cell lines and tissues.

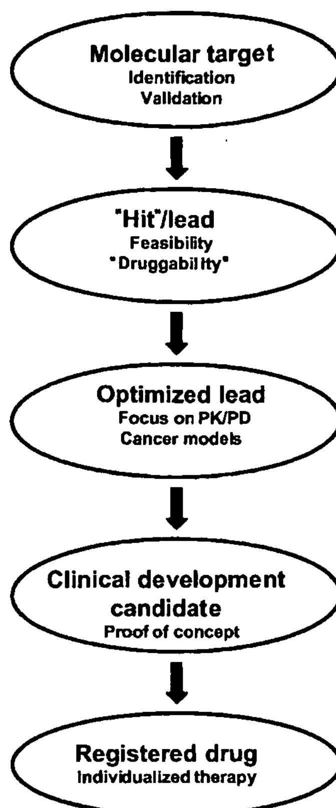
The likely success of this approach is exemplified by the recent unexpected discovery that *BRAF* is an oncogene that is activated in about 70% of melanomas, 10% or more of colorectal cancers, and a smaller subset of other tumors [11]. This exciting finding, made under the auspices of the Cancer Genome Project (Sanger Centre, Hinxton, UK), indicates that the kinase encoded by the *BRAF* oncogene is an excellent target for drug discovery. One possibility is that drugs could be developed that would be selective for the mutationally activated BRAF. Such a drug would be effective in the genomically defined subset of tumors that express and are driven by the mutant kinase gene. This approach would be of particular benefit in metastatic melanoma for which therapeutic options are restricted, especially because the mutation rate is particularly high in this cancer. This discovery illustrates a number of points: (1) the power of a high-throughput genome-based approach in the discovery of new cancer genes and drug targets; (2) the potential for discovering new drugs targeted to a particular molecular pathology; (3) the value of understanding the biological function of the cancer gene and the biochemical pathway in which it operates; and (4) the downstream commercial challenges posed by the development of "niche" drug products that may have high therapeutic value but in a genomically restricted subset of cancer patients [43].

New technologies for drug discovery

Although drugs such as imatinib, trastuzumab, and gefitinib represent major technical achievements, as well as genuine medical advances, in each case there was a considerable delay between the discovery of the target and the regulatory approval of the drug. In the case of imatinib, more than 40 years elapsed between the discovery of the Philadelphia chromosome translocation and the marketing of imatinib. To accelerate drug discovery and patient benefit, the power of a range of effective, often high-throughput technologies is now being harnessed (see Figs 2 and 3).

As already discussed, high-throughput DNA sequencing and associated genomic and bioinformatic techniques are being used to speed up gene discovery and hence the identification of new molecular targets. RNAi technology is proving to be a powerful and simple means of knocking out gene function as part of target validation. Genomic and proteomic technologies are now having an impact across all areas of basic research and drug development. A particular advantage is the large number of genes, mRNAs, and proteins that can be interrogated in a single experiment. For a more extensive recent commentary on this area see Weinstein [41] and Workman [45].

High-throughput screening (HTS) is an extremely effective way of identifying small-molecule "hits" that act on a novel drug target [1]. Large compound collections from tens of thousands up to millions are required for screening campaigns involving biochemical or cell-based assays. Where the structure of the target is known or can be modeled, HTS is complemented by methods such as *in silico* screening of virtual libraries containing millions of "drug-like" compounds against the target of interest, using sophisticated computer algorithms [21]. Fragment-based screening, which involves using X-ray crystallography or nuclear magnetic resonance methods to search for very low molecular weight compounds that show weak interactions with the target, can also be profitable [5]. The use of a combination of these hit-finding methods can be highly synergistic. Following the identification of a screening hit, or more likely a series of hits against a given molecular target, the quality and potential of the hit is evaluated. Practical factors such as physicochemical properties [22], feasibility of synthesis, and overall



- **Basic cell and molecular biology**
- **Molecular oncology**
- **Genomics/genetics**

- **High-throughput screening**
- **Structural biology (x-ray, NMR)**
- **Combinatorial chemistry**

- **Medicinal chemistry**
- **High-throughput PK/ADME**
- **Gene expression microarrays**
- **Proteomics**

- **Molecular PD endpoints**
- **Imaging endpoints (MR, PET)**

- **Pharmacogenomics**

Fig. 2 The impact of new technologies at various stages of the drug discovery process (*PK* pharmacokinetics, *PD* pharmacodynamics, *NMR* nuclear magnetic resonance, *ADME* absorption, distribution, metabolism, and excretion, *MR* magnetic resonance, *PET* positron emission tomography)

"druggability" are important. Combinatorial chemistry and other new chemical methods can be used not only to create chemical diversity for HTS, but also to make more targeted libraries and for "lead explosion" to establish initial structure-activity relationships [15, 36]. Parallel synthesis methodology is valuable at this stage.

Optimization of a selected lead series towards the profile of desired properties is often focused on two main areas: (1) potency and selectivity; and (2) pharmacokinetics and absorption, distribution, metabolism, and excretion (ADME) properties. Robust assays, preferably high-throughput, need to be put in place for all these properties. These assays are formulated into a hierarchical test cascade [1]. Structure-based optimization, for example exploiting the X-ray cocrystal structure of the target–inhibitor complex, can be highly complementary to classical medicinal chemistry-based optimization. An important area for chemical innovation at the interface with bioscience is that of chemical biology [2, 37].

The ability to convert potent and selective lead compounds with activity on cancer cells in culture into agents with robust drug-like properties, particularly in terms of pharmacokinetic and metabolic properties, remains a particular challenge. It is difficult to predict such properties *ab initio*. In vitro ADME methods and higher throughput pharmacokinetic techniques, such as cassette or cocktail dosing, can be extremely valuable when used carefully with suitable lead series [33].

Mechanism of action and pharmacodynamic endpoints

It is absolutely essential during both preclinical and clinical development that particular key milestones are met. Such milestones can often constitute *go/no-go* decision points. As shown in Fig. 4, it is critical to know that active plasma and tissue concentrations of drug can be achieved in animals and patients. Next it is important to demonstrate the desired activity on the intended molecular target (e.g. kinase inhibition), followed by modulation of the corresponding biochemical pathway (e.g. RAS → ERK signaling) and also the achievement of the desired downstream biological effect (e.g. inhibition of proliferation, blockade of angiogenesis, or induction of apoptosis). Finally, these molecular and cellular events need to be linked to the therapeutic response, e.g. tumor cytostasis or regression. It is important that pharmacokinetic/pharmacodynamic relationships are established and that a pharmacological "audit trail" is constructed, consisting of measured parameters for each of the levels of analysis mentioned above (see Fig. 4, and references 46 and 48 for more details).

Pharmacodynamic endpoints may be measured on tumor biopsies or surrogate normal tissue such as peripheral blood lymphocytes, skin or buccal mucosa. Alternatively, and preferably, minimally invasive assays employing techniques such as positron emission

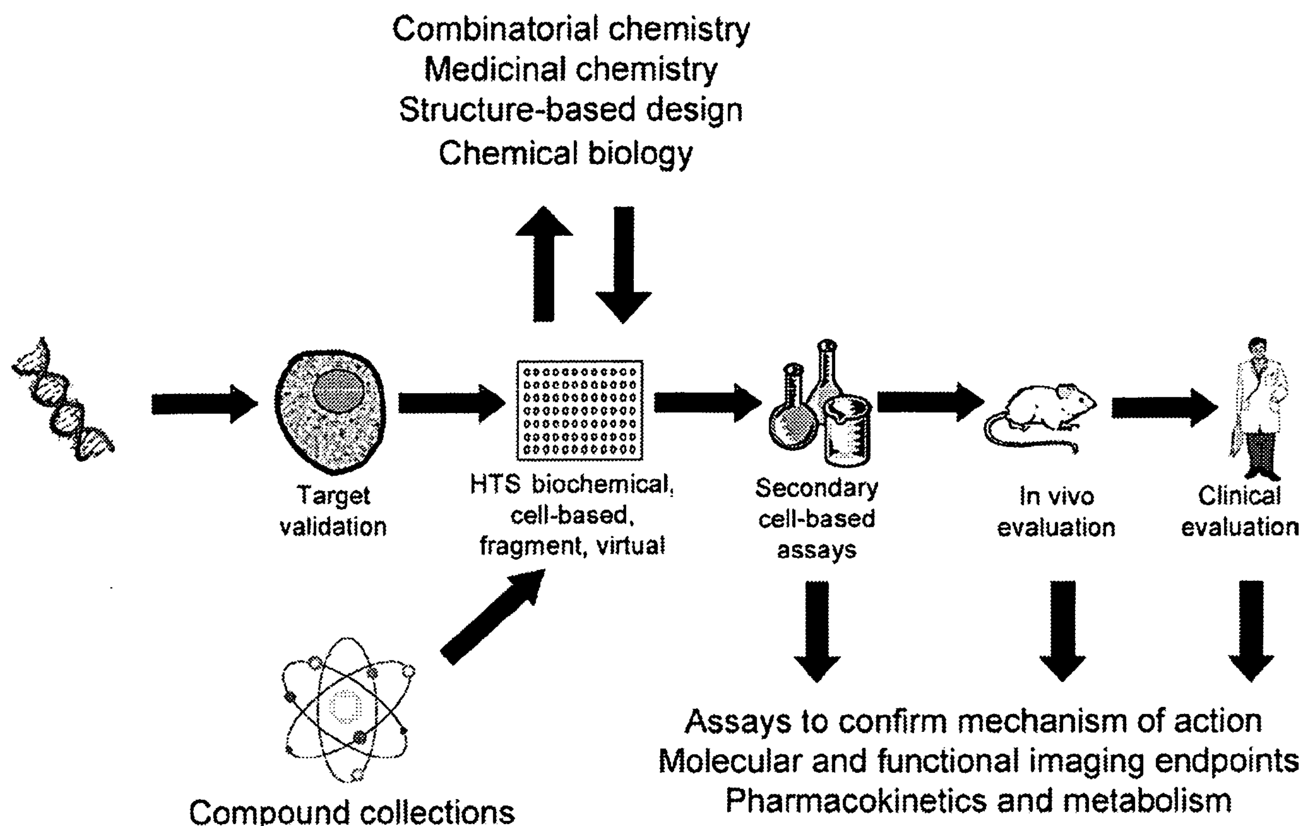


Fig. 3 Process of contemporary drug discovery (*HTS* high-throughput screening)

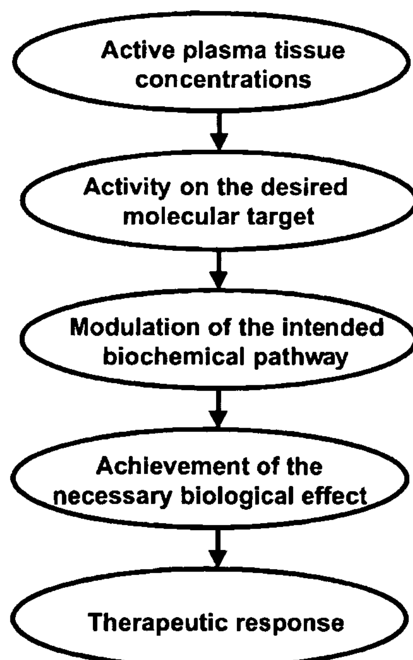


Fig. 4 Key milestones in preclinical and clinical drug development. Measurements made at each milestone allow construction of a pharmacological "audit trail" (see references 45 and 46)

tomography (PET) and magnetic resonance spectroscopy/imaging (MRS/MRI) can be extremely valuable [46, 48].

Invasive molecular endpoints can for example involve changes in protein phosphorylation, as measured by Western blotting, enzyme-linked immunosorbent assay (ELISA), or immunohistochemistry. Genome-wide expression profiling by microarray and also global proteomic analysis can provide a rich source of potential pharmacodynamic endpoints, as well as helping to understand the cellular mode of action of a drug, which may not always be as intended [8, 9, 45].

Current issues in the development of new molecular cancer therapeutics

Although rich in potential and showing signs of considerable promise, the new genome-based approach is not without its challenges (e.g. see references 3, 10, and 43). This is exemplified by the recent clinical trial results with gefitinib [14, 19]. The trials concerned were randomized, double-blind, phase III studies in which gefitinib when used in combination with chemotherapy (gemcitabine and cisplatin or paclitaxel and carboplatin) failed to improve survival in patients with chemotherapy-naive advanced non-small-cell lung cancer (NSCLC). This was perhaps surprising given that gefitinib has activity as a single agent in NSCLC, as well as in head and neck malignancy, and in hormone-refrac-

tory prostate cancer [12]. In addition, studies in preclinical models showed a benefit for the combination of gefitinib with chemotherapy. There are a number of possible explanations for the inability of gefitinib to improve clinical outcome for the particular tumor type and chemotherapy regimens concerned. One is that gefitinib and cytotoxic therapy are each maximally effective against the same tumor cell population; hence there is no additive, let alone synergistic, interaction. Another possibility is that gefitinib may block cell-cycle progression in tumor cells, thereby antagonizing the effects of cytotoxic therapy. These factors presumably outweigh potentially advantageous interactions such as blockade by gefitinib of survival pathways that might be used by cancer cells to protect themselves against cytotoxic damage. It could also be speculated that for some reason, possibly relating to changes in signaling pathways, gefitinib may be more effective in the biological context of previous exposure to chemotherapy.

Of particular potential importance is the possibility that there may be a subset of NSCLC patients who have molecular characteristics that predispose them to be responsive. This may not relate simply to the level of expression of the epidermal growth factor receptor molecular target, but could feasibly correlate with the flux through the receptor tyrosine kinase → RAS → RAF → MEK → ERK1/2 signal transduction pathway (potentially measurable using antibodies to phospho-ERK1/2) or with the expression of any number of genes that could be detected by microarray profiling. Pharmacogenomic analysis is required to identify such genes, and studies of this type will need to be an important part of the future clinical evaluation of gefitinib and other molecular therapeutics. We discuss later in this section the possibility that the optimal use of gefitinib may require a combination involving other molecular therapeutics to take out additional oncogenic pathways in NSCLC and other tumor types.

In the case of trastuzumab, although this agent clearly improves the response of ERBB2-positive breast cancer patients to cytotoxic chemotherapy, when used with anthracyclines it does have significant toxicity [12]. In addition, whereas imatinib is extremely active in the early phase of chronic myeloid leukemia (CML), it produces only short-lived responses in the accelerated and blast crisis stages of the disease; furthermore, acquired resistance to the drug is seen in chronic-phase patients, often due to mutation of the BCR-ABL kinase to a form that is no longer susceptible to imatinib [49].

One of the most important characteristics that may limit the effectiveness of signal transduction inhibitors and other molecular cancer therapeutics is the fact that the malignant progression of most cancers is probably driven by multiple oncogenic defects. Extensive epidemiological data would support the view that 5–7 rate-limiting genes are involved, although there may be as many as 10–12 oncogenic abnormalities in tumors such as pancreatic cancer. The concept of a stepwise accumulation of genetic and epigenetic abnormalities driving malignant progression is probably best exemplified in colorectal cancer [39]. Here, combinatorial oncogenesis involves a conspiracy between mutations in genes such as *RAS*, *APC*, and *P53*, which combine together to accelerate the conversion of normal cells into full-blown invasive and metastatic cancer. Although the precise source and role of genetic instability and its involvement in driving early- versus late-stage malignancy remains a highly controversial issue, there is no doubt that a high level of genetic chaos is a common feature of the major epithelial cancers such as those of the lung, breast, and bowel, as well as in the leukemias, as evidenced by the presence of large-scale amplifications, deletions, and translocations [26]. Genes involved in checkpoint control, mismatch repair, and telomere maintenance may all contribute to genomic instability and the progressive accumulation of cancer-causing defects.

The concept and reality of multistep combinatorial oncogenesis has a number of implications for the development and use of molecular cancer therapeutics. Principle among these is the issue as to whether therapeutic "correction" of a single oncogenic defect will be sufficient to achieve a significant or optimal therapeutic effect—or whether it will in fact be necessary to attend to all or at least several of the key molecular abnormalities to put the brake on combinatorial oncogenesis.

The potential problem is illustrated in Fig. 5. In the particular model example shown (Fig. 5A), the normal cell is transformed into a fully malignant cancer cell by the deregulation of three "mission-critical" pathways, most likely involving the hijacking of normal controls on proliferation signaling, cell-cycle regulation, and survival/apoptosis [13]. Pharmacological modulation of the first pathway, involving genes A–D, is without significant therapeutic effect (Fig. 5B). Similarly, intervention in the second oncogenic pathway, involving genes E–H, also confers little or no therapeutic benefit, either alone or in combination with modulation of the first pathway (Fig. 5C). However, simultaneous intervention in all three oncogenic pathways does have a major therapeutic effect (Fig. 5D). So, the model presented in Fig. 5 would predict that combinatorial oncogenesis would require combinatorial therapy. How do the data stack up against this prediction?

Surprisingly, perhaps, there are a number of published examples in which molecular correction of a single oncogenic abnormality can bring about a therapeutic effect, even in the context of multiple genetic abnormalities [40]. Examples include knockout of oncogenes such as *RAS* or *MYC*, or reintroduction of a lost tumor suppressor gene such as *P53*, *APC*, or *PTEN*. To explain such results, one can invoke the "house of cards" model and the oncogene addiction/tumor suppressor gene hypersensitivity concept [40]. In the house of cards model, the tumor requires each of the molecular abnormalities to power up malignancy; remove any one of the molecular batteries and the cancer cell collapses like a house of cards. In the related oncogene addiction/tumor suppressor gene

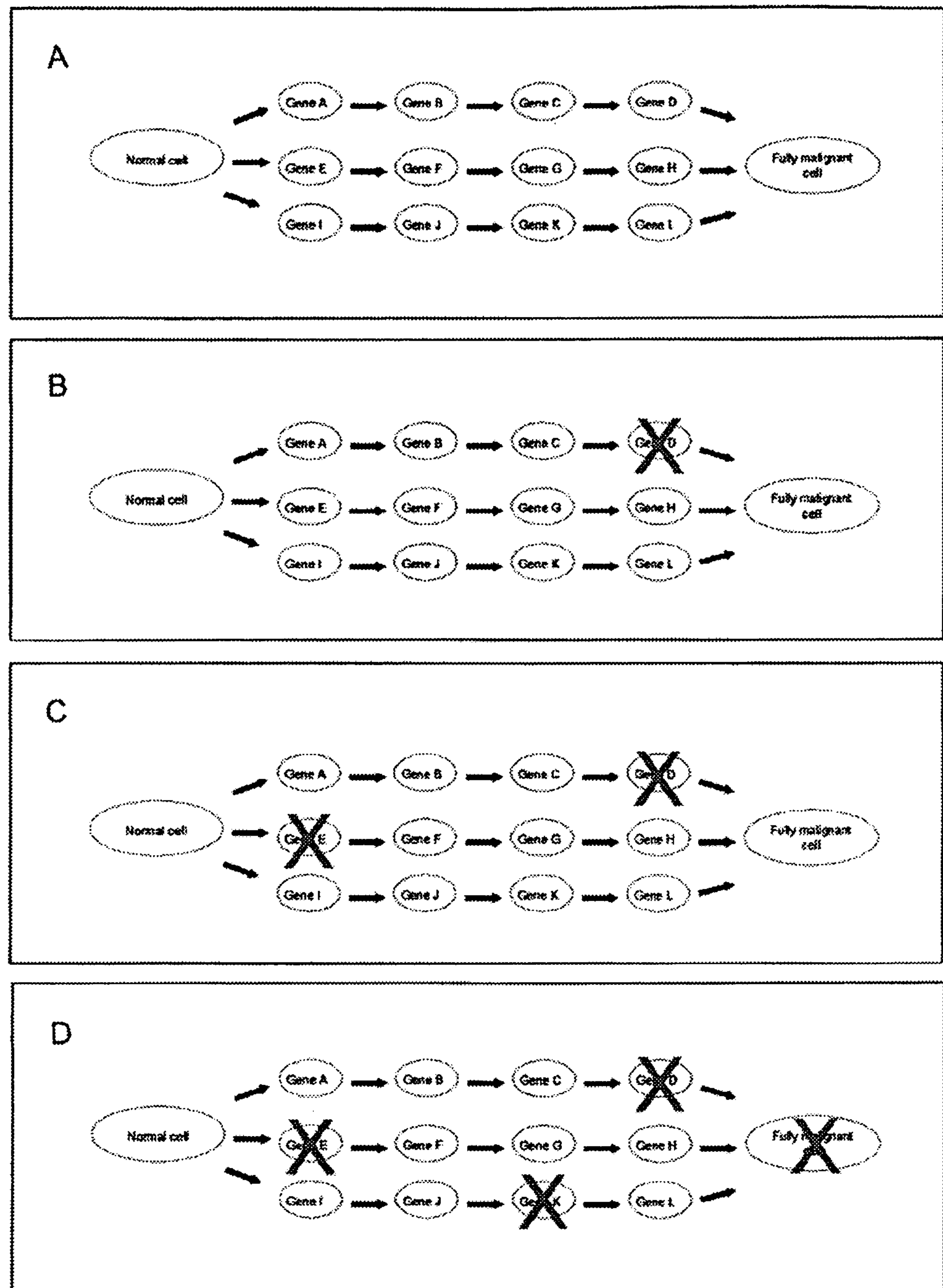


Fig. 5A–D Combinatorial oncogenesis may require combinatorial therapy. In this model, the malignancy is driven by three "mission-critical" pathways. The first pathway comprises the products of genes A–D, the second pathway the products of genes E–H, and the third pathway the products of genes I–L. As shown, the inhibition of one or two of the pathways may be insufficient for a significant therapeutic effect—combinatorial therapeutic blockade of all pathways is required for optimal treatment

hypersensitivity concept, genome instability and selection for malignancy leads to the "hard-wiring" of mission-critical oncogenic pathways and the loss of alternative or redundant signal transduction pathways. As a result, the cancer cell develops a dependence on, or addiction to, the hard-wired oncogenic pathways, together with enhanced sensitivity to reactivation of tumor suppressor functions. Because of this, treatment with a molecular therapeutic that inhibits an activated, hard-wired oncogenic pathway or reactivates a lost tumor suppressor function results in a preferential response in the cancer cell compared with its normal counterpart. It is clearly possible to invoke the oncogene addiction model to explain why a selective anticancer effect can be obtained with molecular cancer therapeutics that hit signal transduction pathways that are activated in cancer cells but that are also important for normal cell function. Probably the best example of this is the selective activity of mTOR inhibitors [e.g. rapamycin (sirolimus) derivatives] and PI3 kinase inhibitors (e.g. LY2940022) against cancer cells that have lost PTEN tumor suppressor gene function, thereby activating the PI3 kinase–AKT–mTOR pathway [27].

How does the clinical experience fit with the oncogene addiction model and the need for the correction of single versus multiple molecular abnormalities? The activity of imatinib in chronic-phase CML and gastrointestinal stromal tumors can be cited as supporting the oncogene addiction model. It is likely, however, that these are cancers in which only a single genetic defect is driving malignancy, i.e. *BCR-ABL* and mutant c-*KIT* respectively. Indeed, the lower activity in imatinib in acute and blast-phase CML and also in acute lymphocytic leukemia, where additional mutations are present, supports the view that combinations of agents may be needed to block these multiple defects. A similar argument can be made to account for the partial, although usually incomplete, responses that are seen with other molecular cancer therapeutics such as trastuzumab and gefitinib. It appears possible, then, that oncogene addiction to a single hard-wired, mission-critical pathway is partial rather than absolute. Oncogene addiction may well be present but in most cases there may be overlapping dependence on several genes and pathways. If this is correct, it would follow that treatment with a targeted drug cocktail would be advantageous. In addition, this would be likely to decrease the likelihood of resistance arising to a single agent, as seen in the clinic with imatinib in CML. This is entirely analogous to the use of multiple drug cocktails in HIV/AIDS. On the other hand, as we target several oncogenic pathways that are also used by normal cells, the key question then becomes: can we retain a therapeutic window between malignant and normal cells?

The development of HSP90 inhibitors

Given the above discussion on the likely advantage of a combinatorial blockade of multistep oncogenesis, the development of HSP90 inhibitors is brought into particularly sharp focus. The factors contributing to the "credentialing" or validation of HSP90 as a therapeutic target, together with the likely advantages of this therapeutic approach, are summarized in Table 2. HSP90 is not a product of a cancer gene per se but rather it is a protein that is required for the malignancy-driving properties of a number of bona fide oncogenes [24, 29].

The HSP90 family comprises HSP90α, HSP90β, the endoplasmic reticulum homologue GRP94, and the mitochondrial counterpart TRAP1. HSP90 is a molecular chaperone involved in protein folding. It is not, however, a generic chaperone that is required for the folding of cell proteins. Nor is it only involved under stress conditions such as heat shock. Rather, it is responsible under normal cellular conditions for the later stage folding and maintenance of the correct conformation and functional activity of a relatively restricted selection of "client" proteins. Many of the clients on this "celebrity A list" have oncogenic activity. They include several oncogenic kinases such as ERBB2, RAF-1, CDK4, POLO-1, and MET. In addition, HSP90 clients also include mutant P53, HIF-1α, estrogen/androgen receptors, and the catalytic component of telomerase hTERT. Thus inhibition of HSP90 activity leads to incorrect folding and subsequent degradation by the ubiquitin-proteasome pathway of all the above-mentioned oncogenic clients. As a result, HSP90 inhibitors are likely to block all six of the so-called "hallmark traits" of malignancy [16] and therefore have potential for one-step combinatorial therapy against a broad range of cancers. Furthermore, based on the work of Lindquist and colleagues [35], it might be speculated that inhibition of HSP90 could uncover synthetic lethal mutations in cancer cells.

Encouragingly for the approach, certain natural products that were known to have anticancer activity were found to target HSP90 [24, 29]. In particular, these include radicicol and geldanamycin (see Fig. 6 for chemical structures). These agents work by competing

Table 2 HSP90 target validation (for further details see reference 24)

Molecular chaperone involved in protein folding
Overexpressed in human tumors (e.g. due to stress and oncoproteins)
Essential for stability and function of many oncogenic "client" proteins e.g. ERBB2, RAF-1, CDK4, POLO-1, MET, mutant P53, HIF1α, estrogen/androgen receptors, and telomerase hTERT
Inhibition likely to block all six "hallmark traits" of cancer
Potential for one-step combinatorial therapy against a broad range of malignancies
May uncover synthetic lethal mutations in cancers
Natural products that target HSP90 have anticancer activity
Proof of concept for therapeutic selectivity demonstrated in human tumor xenograft models
First-in-class inhibitor 17AAG now showing evidence of biological and clinical activity at well-tolerated doses

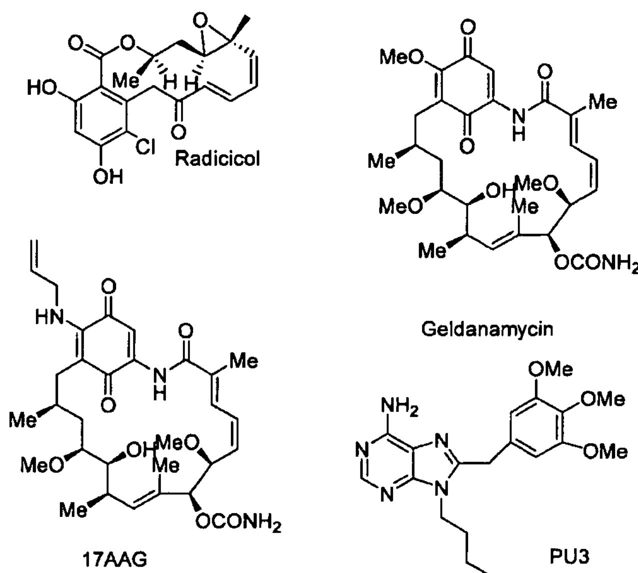


Fig. 6 Chemical structures of HSP90 inhibitors

with ATP for binding at the nucleotide-docking site located in the N-terminal domain of HSP90 [32, 34]. ATP binding and hydrolysis are essential for the functioning of the chaperone and drug binding prevents the correct assembly of mature HSP90/client protein/cochaperone complexes. This appears to result in recruitment of a ubiquitin ligase to the immature complex, leading to proteasomal degradation of client protein [24].

Proof of concept for therapeutic selectivity towards cancer cells was exemplified with the geldanamycin analog 17AAG (Fig. 6) in human tumor xenograft models grown in immunosuppressed mice [20]. Furthermore, 17AAG has entered clinical trials as the first-in-class inhibitor of HSP90 and is now showing consistent molecular evidence of the desired mechanism of action, together with early indications of therapeutic activity [4].

We have shown that treatment of human colon cancer cells with 17AAG leads to combinatorial depletion of key oncogenic client proteins such as RAF-1 and AKT, consistent with the demonstrated inhibition of the ERK1/2 and PI3 kinase signaling pathway and the downstream induction of cell-cycle arrest and apoptosis [8, 17].

We have used global gene expression microarray profiling to investigate genes that might be involved in sensitivity to 17AAG, as well as to identify potential pharmacodynamic markers of effective HSP90 inhibition [8]. In addition, we used proteomic analysis to identify global responses to HSP90 inhibition by 17AAG at the protein level (collaboration with Professor Mike Waterfield and colleagues, Ludwig Institute for Cancer Research, University College London, London, UK). A molecular signature of HSP90 inhibition has been defined, consisting of depletion of client proteins such as RAF-1, CDK4, and ERBB2 at the protein level (with no effect at the mRNA level) together with upregulation of HSP70 at both the mRNA and protein levels [24]. In some cancer cell lines, HSP90 itself is upregulated. We routinely determine the molecular signature of HSP90 inhibition by Western blotting. In addition, we are also developing ELISA assays for greater sensitivity and more straightforward quantification.

In terms of the expression of genes that may confer sensitivity or resistance, we have shown that high levels of the quinone reductase NQO1/DT-diaphorase cause considerable sensitization toward 17AAG, which has a 17-allylamino group, although not to the major metabolite of 17AAG, which has an amino moiety at the 17 position, or to geldanamycin, which has a methoxy group at the 17 position [20]. The results suggest a role for activation via quinone metabolism, although the HSP90 mechanism is retained. Further work is required to elucidate the details and full significance of the effect.

Interestingly, our studies have also suggested that tumor lines that respond to treatment by expressing increased levels of the HSP90 target itself may recover more rapidly from the effects of 17AAG and therefore be less sensitive to the drug [8].

In collaborative studies published recently, we have identified the new gene product AHA1 as a novel cochaperone that activates the essential ATPase activity of HSP90 and which is upregulated in human tumor cells by stress, heat shock, and pharmacological HSP90 inhibitors [30]. Using a combination of gene expression microarrays, proteomics (two-dimensional gel electrophoresis with MALDI mass spectrometry) and Western blotting, we showed that *AHA1* gene expression is upregulated at the level of both mRNA and protein in response to treatment of human tumor cells with the HSP90 inhibitors radicicol and 17AAG. The mechanistic, pharmacological, and therapeutic significance of these observations is now under investigation.

Having shown good activity in xenograft models and an acceptable therapeutic index in animal models, 17AAG has been taken into clinical trials in our own institution and at our four centers in the USA under the auspices of the US National Cancer Institute and Cancer Research UK (formerly the Cancer Research Campaign). In the UK trial at the Cancer Research UK Centre for Cancer Therapeutics, Institute of Cancer Research, and the Royal Marsden Hospital [4], 17AAG has been given weekly by intravenous infusion at doses up to 450 mg/m^2/week. Pharmacokinetic studies show that plasma concentrations are above the IC_{50} for inhibition of tumor cell growth for prolonged periods. In addition, depletion of RAF-1, CDK4, and the SRC family kinase LCK has been clearly demonstrated in peripheral blood lymphocytes, together with upregulation of HSP70. Furthermore, depletion of RAF-1 and CDK4 alongside increased expression of HSP70 has also been observed in malignant tissue by comparing tumor biopsies taken before and after treatment. Consistent with these molecular changes, we have seen evidence of disease stabilization in some patients. RNA has been prepared from certain tumor biopsies to allow global expression profiling to be carried out. This should generate valuable results to compare with those from in vitro cell-culture exposures [8].

Although relatively invasive assays are providing valuable information by demonstrating that 17AAG is able to inhibit its molecular target both in peripheral blood lymphocytes and in tumor biopsy material, minimally invasive assays such as those involving PET and MRS/MRI would have major advantages [46, 48]. In collaboration with Professors Martin Leach, John Griffiths, and colleagues (Cancer Research UK Biomedical Magnetic Resonance Group, St George's Hospital Medical School, London, and Cancer Research UK Clinical Magnetic Resonance Research Group, Institute of Cancer Research and Royal Marsden Hospital, Sutton, UK), we have noted interesting changes in human xenograft tumors following treatment with 17AAG, in particular an unusual increase in the levels of phosphoethanolamine and phosphocholine [7]. These may be indicative of alterations in lipid signaling and/or membrane turnover. In addition, we are collaborating with Professor Pat Price and Dr. Eric Aboagye (Cancer Research UK PET Oncology Group, Molecular Imaging

Centre, Manchester, and Cancer Research UK PET Oncology Group, MRC Cyclotron Unit, Hammersmith Hospital, Imperial College School of Medicine, London, UK) to use labeled choline PET tracers to monitor the effects of 17AAG in tumors [23]. Overall, the potential to use molecular or functional imaging to monitor the pharmacodynamic effects of the new molecular cancer therapeutics is an exciting area.

17AAG shows significant promise and demonstrates proof of concept for HSP90 inhibition in humans. It does, however, have a number of potential limitations. These include:

- Limited stability and complex formulation
- Modest potency against the HSP90 target
- Substrate for P-glycoprotein
- Activated by polymorphic NQO1/DT-diaphorase
- Metabolism by polymorphic cytochrome P450
- Low oral bioavailability
- Limited therapeutic index

Because of these potential issues, several groups are seeking small-molecule, synthetic inhibitors of HSP90 as alternatives to the existing natural products. A range of approaches are likely to be taken, including those described earlier in this commentary and depicted in Fig. 3.

One interesting lead that has emerged is the synthetic purine-based compound PU3 (Fig. 6). This agent has been shown to inhibit HSP90 in cancer cells and to retard their growth [6]. PU3 appears to behave like the natural product agents, competing with ATP at the nucleotide-binding site of the N-terminal domain of HSP90 [6]. Another interesting compound is novobiocin. This appears to act in a different way by binding to the C-terminal domain of HSP90 [25]. Given the attractiveness of the target and the encouraging results with 17AAG, it appears likely that more synthetic chemical inhibitors of HSP90 will emerge.

There are many challenges ahead with HSP90 inhibitors. Some of the important outstanding questions include:

- What is the optimal treatment regimen?
- How should the drug be used as a single agent?
- How should the drug be used in combination with cytotoxics, e.g. paclitaxel [28]?
- Will any tumor types be particularly sensitive?
- Are any particular client proteins especially important for response in certain tumor settings?
- Will particular genomic abnormalities predispose to sensitivity or resistance?

Conclusions

The following overall conclusions can be drawn:

- Proof of principle is now established that targeting cancer genome abnormalities and the molecular pathology of cancer can be clinically beneficial.
- New molecular targets continue to emerge from cancer genomics.
- Blocking multistep oncogenesis will most likely require combinatorial therapies.
- This may be delivered in individualized cocktails of molecularly targeted agents.
- HSP90 inhibitors such as 17AAG may block multiple oncogenic pathways in a single drug.
- Deployment of multidisciplinary skills and new technologies is required to accelerate the pace and improve the efficiency of drug discovery against new molecular targets.
- Clinical development strategies must pay close attention to the proposed mechanism of action and a pharmacological audit trail must be constructed to allow rational decision-making, including *go/no-go*.
- Demonstration of proof of concept is invaluable in hypothesis testing phase I clinical trials.
- Pharmacodynamic and pharmacogenomic markers are essential for success.

The explosion of new molecular targets and the development and application of many powerful technologies should accelerate the discovery of innovative molecular therapeutics. There are many challenges ahead and the risks associated with each individual agent remain considerable, but the prospects for overall success with individualized therapies targeted to the molecular pathology of the individual patient are excellent [47, 49]. This exciting translational work requires many disciplines (e.g. chemistry, biology, and medicine) and organizations (e.g. academia, biotech, and large pharmaceutical companies) to work together internationally to accelerate patient benefit.

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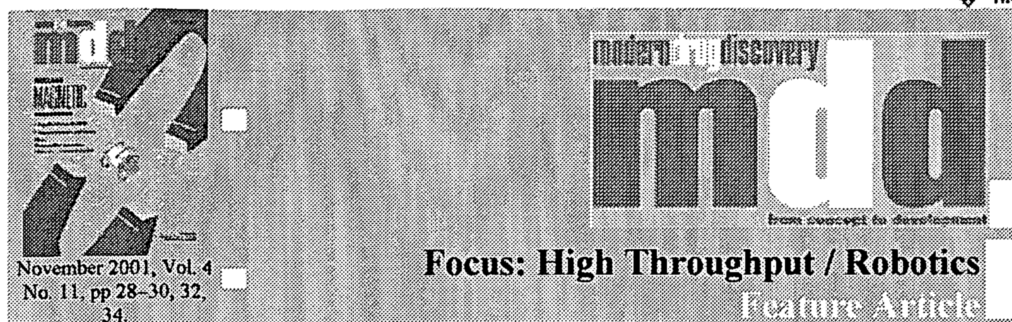
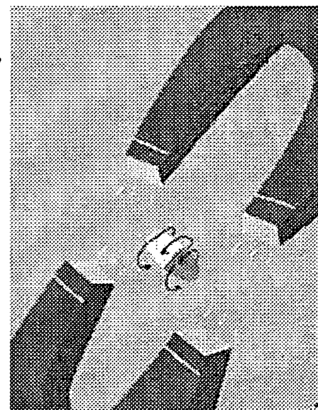


Screening with NMR

DAVID BRADLEY

Advances in NMR automation have allowed researchers to follow drug development from beginning to end.



Nuclear magnetic resonance (NMR) spectroscopy has been widely adopted since its invention. What was once a cumbersome technique can now reveal the most cryptic details of sophisticated molecular systems. It is one of the most information-rich analytical techniques. The latest machines can place very small samples in a magnetic field with a strength of more than 21 T and detect radio-frequency signals of almost 1 GHz. Systems capable of automated and high-throughput sampling are poised to push NMR into the mainstream, not just as the analytical tool of choice but as a key component of the drug discovery process.

NMR speeds up

Several research teams are working on bringing NMR spectrometers into drug discovery laboratories and using them to further accelerate the rate of pharmaceutical R&D. According to researchers at Varian (Palo Alto, CA), one of the serious drawbacks in getting the best results from a combinatorial array is the inability to obtain a complete sample analysis.

In pioneering work on LC-NMR carried out by Jeremy Nicholson and John Lindon at Imperial College (London), in collaboration with Manfred Spraul of Bruker GmbH, Nicholson's team separated and assigned a randomly synthesized collection of 27 tripeptides—all the combinations of Ala, Tyr, and Met—using one chromatographic run that took about 30 min (*1*). In Nicholson's words, "Not a bad first attempt!" Varian scientists recently extended Nicholson's research to other areas of combinatorial chemistry by devising an automated approach to NMR that allows combinatorial chemists to quickly and easily obtain the ^{1}H-NMR

spectra of solution-phase samples.

The Varian team worked on obtaining the NMR spectra of compounds bound to solid supports and was rewarded with the rapid adoption of its techniques throughout the combinatorial community. Unfortunately, the teams' solid-state NMR approach is confined to analyzing small numbers of samples and lacks the high-throughput capability needed for efficient analysis of vast compound libraries. A flow technique coupled with automated sample analysis using liquid-phase NMR would help the analyst rein in combinatorial libraries.

While developing HPLC-NMR techniques, the Varian team realized that the LC-NMR approach could be refined as a useful tool for combinatorial applications. Combinatorial chemistry not only traditionally generates large numbers of compounds in small quantities, but also tends to do away with the use of conventional glassware, replacing it with the increasingly familiar multiple-welled microtiter plates. To address these issues, Varian scientists built and tested a flow-NMR sample changer. "The system reduces the cost, time, and effort of sample handling, allows inexpensive sample containers to be used, and uses smaller quantities of sample than traditional automated NMR systems," according to Varian.

The flow-NMR approach precludes the need for transferring samples from the microtiter plates to NMR tubes, which would be the biggest cost in high-resolution NMR of a large library, for which not only precision glass tubes and deuterated solvents are required for each sample from each cell, but also a drying (solvent removal) process. Instead, the team at Varian used an automated liquid-handling device, such as the Gilson Model 215 Liquids Handler, which takes a sample solution stored in a microtiter plate and injects it directly into an NMR flow probe.

With each step of the protocol controlled by a computer, the system first rinses the NMR flow cell with a solvent and disposes the waste solvent. The liquid handler then moves a controlled volume of the appropriate sample into the NMR probe, at which point the spectrometer is signaled to begin gathering data. The process can be repeated automatically with any number of NMR experiments on each sample. The team refers to the approach as direct injection (DI) NMR; and the liquid handler is referred to as the versatile automated sample transport (VAST).

The DI VAST approach can quickly gather one-dimensional ^{1}H-NMR spectra for each member of a combinatorial library, an approach that the team says is almost routine at Varian and elsewhere (Figure 1). For example, at Monsanto (St. Louis) Bruce Hamper and his team used the VAST system to characterize a 96-member substituted methylene malonamic acid library (2).

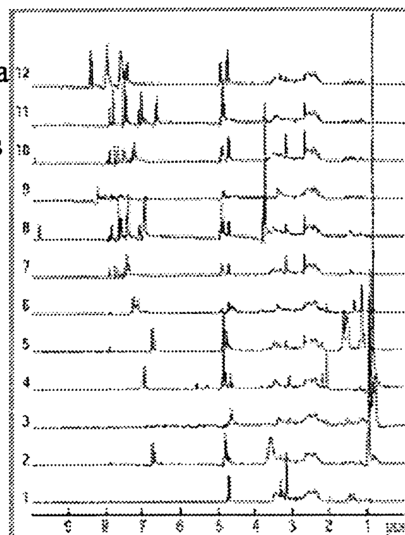


Figure 1. Seeing how things develop. Using automated systems developed by companies such as Bruker and Varian, researchers can quickly generate ^{1}H-NMR spectra of compounds synthesized in a 96-well plate. (Adapted from Reference 2.)

"This only works in libraries that have one compound per well," points out Lenore Martin, assistant professor in the department of biochemistry, microbiology, and molecular genetics at the University of Rhode Island. The standard in the industry is to have groups of compounds in each well, so there is still a requirement to couple the flow cell to a separation technique such as LC. "Another very promising technique is capillary electrophoresis (CE)-NMR," adds Martin, "which is being developed by a group in the department of chemistry at the University of Illinois, Champaign-Urbana."

Drug design by NMR

NMR is ideal for screening fragments of potential drug molecules, according to the work of Stephen Fesik of Abbott Laboratories (Abbott Park, IL). Recently, he and his colleagues devised a strategy for designing high-affinity ligands to create drugs that inhibit kinases (3). Fesik says that finding leads of sufficient specificity, bioavailability, and safety is "still an arduous process" and usually has a failure rate of 50% in the initial stages of drug discovery. A method to bump up successes without added synthetic effort would be useful. Fesik's "fragment" approach fits the bill and involves screening a range of fragments that could be incorporated into an inhibitor without reducing potency but improving characteristics, such as solubility or reduced toxicity.

The first step is to fragment an existing lead molecule, identify a range of suitable replacements for the fragments, and build these into the original molecular skeleton. The problems arise in trying to identify suitable fragments. The fragments bind weakly to the target receptor or enzyme, so conventional screening methods cannot reliably detect their binding, because high concentrations are required to generate a detectable response. Moreover, standard assays indicate nothing about binding orientation or site, and so they offer no clues about optimal positioning of the fragment on the skeleton.

Fesik and his colleagues found a way to screen such fragments

successfully by using NMR based on a Bruker system. The affinity and binding site location of the chosen fragment are determined by watching how the ^{15}N–^{1}H heteronuclear single quantum coherence (HSQC) spectra of the ^{15}N-labeled protein change when the test molecule is added. The next step involves using NMR to identify molecules that bind to the same site as the chosen fragment. The fragments identified can then be incorporated into the skeleton for further study.

"This approach is a valuable strategy for modifying existing leads to improve their potency, bioavailability, or toxicity profile, and thus represents a useful technique for lead optimization," says Fesik. Moreover, he emphasizes that the use of NMR in this manner means that thousands of potential mimetics with a range of functionality can be quickly analyzed without the need for multiple synthetic routes to be implemented and thousands of putative leads prepared. Indeed, the Fesik team previously demonstrated high-throughput NMR that could investigate potential ligands for unknown proteins at a rate of 200,000 per month (*4*).

Toward proteomics

If NMR is going to respond to the postgenomic challenge of addressing thousands of new drug targets, innovations are needed to remove two key limitations. First, NMR structural studies cannot be performed for proteins much larger than 35 kD. Second, to attack thousands of proteins, a proteomically leveraged, highly parallel strategy to drug design is needed; but current strategies attack one target at a time. Triad Therapeutics in San Diego is removing both of these barriers, thus extending NMR drug discovery efforts in a proteome-wide manner.

Triad developed a suite of NMR technologies that allow for the characterization of protein–ligand interactions with unprecedented speed (days as opposed to months). These tools, combined with bioinformatics strategies, allow the systematic gathering of information that describes protein–ligand interactions across large gene families of proteins such as kinases and dehydrogenases. The term "enzyme mechanomics" describes this newly enabled gene-family-wide characterization of structure–function correlations.

"Triad makes use of a technology called NMR SOLVE—structurally oriented library valency engineering—to guide the design of combinatorial libraries tailored to entire gene families of proteins, using the enzyme mechanomic data," says Daniel Sem, Triad's vice president of biophysics. He and colleague Maurizio Pellecchia point out that NMR is intrinsically a noninvasive technique and thus is ideally suited to observing the dynamics of a molecular system, as well as acting as an analytical tool.

"Any NMR method that provides structural information on large proteins must provide a way to simplify NMR spectra—to focus in on that part of

a spectrum corresponding to atoms that are in a protein's binding site," explains Sem. As such, Sem, Pellecchia, and colleagues at the University of Wisconsin have devised a technique that can reduce overlap in protein spectra and allow these complex biomolecules to be investigated in their native state with much greater clarity (5). This method, called solvent-exposed amides with transverse relaxation-optimized spectroscopy (SEA-TROSY), is combined with other experiments to look at very large protein structures, their backbone dynamics, and how ligands or inhibitors bind to them.

"NMR is now poised to tackle the postgenomic challenge of attacking large numbers of new drug targets with greater speed, in a highly parallel manner, and without the usual limitation to low-molecular-weight proteins," adds Sem.

The metabolic end point

One approach to drug research closely considers the end product of the drug cycle. Jeremy Nicholson uses high-resolution NMR to screen body fluids and magic-angle spinning NMR to screen tissues for metabolic byproducts of drugs and to detect perturbations in endogenous metabolic profiles in disease processes (6, 7). Nicholson and his colleagues have spent the past two decades looking into metabonomics, a field driven mainly by NMR spectroscopy. Nicholson describes metabonomics, a term he coined about six years ago, as the "quantitative measurement of the dynamic multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification."

Rather than focusing on single analytes as might be the case in a clinical diagnostics approach, Nicholson's team has used ^{1}H-NMR to build up expertise in the multicomponent metabolic composition of cells, tissues, and biological fluids (saliva, blood, urine, semen, and even sweat). The team uses pattern recognition, expert systems, and related bioinformatic tools to interpret and classify the complex data sets generated by one- and two-dimensional NMR analysis of such samples. They can now spot telltale metabolic fingerprints in NMR spectra. NMR, in particular, gives a very complex fingerprint of a large number of metabolite signatures—thousands in the case of a urine sample (Figure 2).

"The quantitative analysis of such profiles gives insight into sites and mechanisms of toxicity according to the characteristic perturbations in the metabolic profile," explains Nicholson. "Biomarker information can be statistically extracted from spectra and, as NMR is a structural organic chemistry tool, novel metabolic markers can be structurally characterized.

"The recovery of high-density metabolic information from complex spectra is facilitated by the use of an array of multivariate statistical and pattern recognition tools that classify toxicity or disease state according to spectral profile and identify critical regions of the NMR spectral fingerprints that are modified by the pathological process," says

Nicholson. Exact biomarker identification is then achieved or confirmed by judicious use of multidimensional NMR spectroscopy (e.g., ^{1}H-^{13}C HSQC or heteronuclear multiple-bond correlation spectroscopy) combined with HPLC–NMR–mass spectrometry methods (8).

A holistic picture

The London team also recently introduced the concept of "integrated metabonomics". This, Nicholson says, is the parallel NMR investigation of multiple biological fluids, and sometimes selected tissue samples, using magic-angle spinning NMR methods at various time points after drug exposure to gain a holistic picture of a series of metabolic events in the whole body.

Nicholson and his colleagues are now involved in cross-correlating integrated metabonomic data with those generated by genomics and proteomics (what he terms "integrated bionomics") to describe the biochemical consequences of pathological processes at multiple levels of biomolecular organization and to learn about silent gene function.

From humble beginnings as a simple spectroscopic tool for working out molecular structures, NMR has raced to the front of the drug discovery arsenal, providing pharma researchers with a powerful weapon with which to hack through the molecular jungle.

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Return to Top || **Table of Contents**

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Biophysics

Plant histochemistry by correlation peak imaging

(plant physiology/nuclear magnetic resonance/*Ricinus communis* seedlings)

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Communicated by Richard R. Ernst, Eidgenossische Technische Hochschule Zentrum, Zurich, Switzerland, August 29, 1995

ABSTRACT **Using a new NMR correlation-peak imaging technique, we were able to investigate noninvasively the spatial distribution of carbohydrates and amino acids in the hypocotyl of castor bean seedlings. In addition to the expected high sucrose concentration in the phloem area of the vascular bundles, we could also observe high levels of sucrose in the cortex parenchyma, but low levels in the pith parenchyma. In contrast, the glucose concentration was found to be lower in the cortex parenchyma than in the pith parenchyma. Glutamine and/or glutamate was detected in the cortex parenchyma and in the vascular bundles. Lysine and arginine were mainly visible in the vascular bundles, whereas valine was observed in the cortex parenchyma, but not in the vascular bundles. Although the physiological significance of these metabolite distribution patterns is not known, they demonstrate the potential of spectroscopic NMR imaging to study noninvasively the physiology and spatial metabolic heterogeneity of living plants.**

In the tissue of plant organs, enzymatic reactions and metabolic pathways are compartmentalized. A striking example is C4-photosynthesis, where the different reaction steps are spatially separated between mesophyll and bundle sheath cells (1). However, the knowledge about the distribution and the concentration of metabolites in plants is still very limited, mainly because of the lack of appropriate experimental techniques. Only a few methods are available to study the localization of metabolites in plant materials. Enzyme localization is accessible by methods of molecular biology—for example, by cDNA *in situ* hybridization and immunohistochemistry, by tissue print (2), or by measuring the activity of β-glucuronidase (3). Extraction of cell sap by microcapillaries is possible only from relatively large cells located close to the surface of the plants (4). Fixation procedures in microautoradiography (5) and electron-dispersive energy-loss spectroscopy (6) of water-soluble compounds or elements might disturb the spatial distribution of metabolites. All of these methods have in common an invasive or even destructive way of measuring the spatial distribution of the constituents of the tissue.

Nuclear magnetic resonance (NMR) measurements are noninvasive by nature. NMR imaging, which is based on the NMR signals from the hydrogen in water molecules, has had an enormous impact on medical diagnostics by visualizing human anatomy in great detail. NMR spectroscopy can provide information on the different chemical constituents in a sample by detecting slight shifts of their resonance frequencies and is widely used in analytical chemistry. The combination of NMR imaging and spectroscopy resulted in a technique known as "chemical-shift imaging (CSI)" (7, 8). CSI enables the spatial distribution of specific chemical compounds within a heterogeneous sample to be measured. Initial applications of CSI to plants have already provided some insight into the spatial distribution of metabolites (9, 10). Being inherently noninvasive, these NMR measurements fully preserve the integrity of the plant. They affect neither its physiology nor the concentrations of the metabolites *in situ*. Therefore, NMR imaging and spectroscopy applied to study plant materials may yield valuable information that cannot be obtained by using any conventional, destructive method.

In data acquired by normal CSI with one spectral dimension, it is sometimes impossible to differentiate between components with overlapping resonance lines. This problem arises particularly in ^{1}H-NMR spectroscopy with its inherently limited spectral dispersion. By using two-dimensional (2-D) correlation NMR spectroscopy (11), the spectral resolution and consequently the information content of the spectra can be improved considerably. Correlation spectroscopy and other multidimensional spectroscopic techniques are already a standard tool in analytical chemistry and in the study of protein structure. First *in vivo* applications of correlation spectroscopy in animals were reported recently (12, 13). In these experiments, specific molecules are identified by their characteristic correlation peaks (i.e., their off-diagonal resonances in a 2-D frequency map, indicating scalar coupled spins within the molecule). Fig. 1 shows a two-dimensional correlation map obtained *in situ* in a plant seedling and demonstrates the wealth of information available with this technique. A large number of chemical constituents including sugars and amino acids and even various anomers can be observed, representing the average concentration of these substances in the examined cross-section of the stem.

For further localization within the plant, we have added phase-encoding gradients to correlation spectroscopy (14) to obtain a correlation-peak imaging (CPI) experiment with two spatial and two spectral dimensions.¶ From the acquired data, a complete 2-D correlation map can be reconstructed for each volume element, showing the metabolite pattern at that location. Furthermore, the spatial distribution of specific metabolites can be visualized by displaying the spatially varying intensity of the corresponding correlation peaks. These "metabolic images" represent the distribution of the metabolites, with the assumption of uniform metabolite relaxation times in all plant tissues. Conventional ^{1}H-NMR images of the plant with high spatial resolution can be acquired in the same experimental setup and allow the

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Abbreviations: CPI, correlation-peak imaging; 2-D, two dimensional; CSI, chemical-shift imaging.

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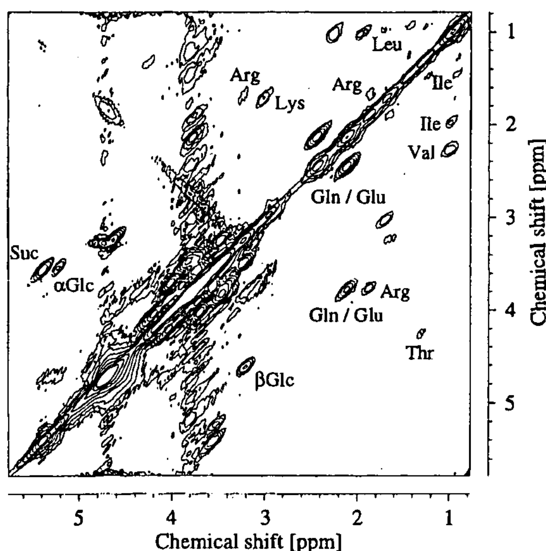

FIG. 1. NMR 2-D correlation spectrum obtained from a 4-mm slice selected *in situ* in the hypocotyl of a 6-day-old castor bean seedling. Most interesting are the spots appearing off the diagonal: the positions of these correlation peaks are characteristic of specific molecules and originate from spins presenting scalar couplings to neighboring spins in the same molecule. From their position, the correlation peaks can be assigned to specific substances (Suc, sucrose; Glc, glucose; and amino acids indicated by their standard three-letter code); even two anomers of glucose can be distinguished. This is the global spectrum of the slice selected in the hypocotyl without further localization, representing the average amount of the detected metabolites in this volume. The goal of the CPI experiment is to measure the spatial distribution of these substances within the slice.

correlation of the measured metabolic distributions with the anatomy of the plant.

METHODS

One of our first attempts to demonstrate the potential of the CPI technique was to measure the spatial distribution of the most abundant carbohydrates and amino acids (sucrose, α- and β-glucose, glutamine/glutamate, arginine, lysine, and valine) in the hypocotyl of a 6-day-old castor bean seedling (*Ricinus Communis L.*) (16). Seedlings were grown in darkness on top of glass tubes fitted into a standard microimaging NMR probe. Thus, the plant could be placed into the spectrometer without disturbing its physiological environment. All experiments were performed on a Bruker (Karlsruhe, Germany) model AMX500 NMR spectrometer, equipped with an 89-mm bore, 11.75-T superconducting magnet, and a shielded imaging gradient system. Both ^{1}H-^{1}H-CPI experiments and conventional NMR imaging experiments with high spatial resolution were conducted for every plant.

RESULTS

The results for one plant are shown in Figs. 2 and 3. In the high-resolution proton image of the hypocotyl anatomy (Fig. 2), eight vascular bundles, the pith parenchyma, and the cortex parenchyma can be seen. The phloem and the xylem, which are important for sucrose and water transport, respectively, can be clearly distinguished within the vascular bundles. Experimental parameters for this microscopic NMR image with a nominal spatial resolution of 24 μm are given in the figure caption.

The metabolite images obtained with the CPI experiment are presented in Fig. 3. They show the distribution of sucrose,

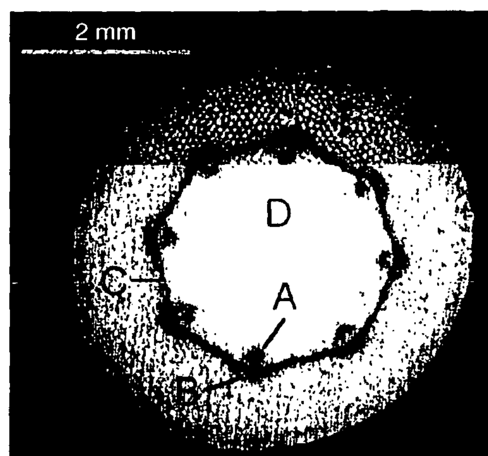

FIG. 2. High-resolution proton NMR image of a cross section of the hypocotyl, which allows the anatomy of the plant to be identified in great detail. Each of the eight vascular bundles consists of the xylem region (A) at the inner side and the phloem region (B) at the outer side of the meristem ring (C). The cellular structure of pith (D) and cortex parenchyma (E) is clearly visible. Cell wall material appears dark. This inversion recovery spin echo image was acquired in 49 min with an inversion delay of 750 msec, an echo time of 8 msec, and a repetition time of 5.75 sec. The 256 × 256 image matrix with a field of view of 6 mm × 6 mm and a slice thickness of 1 mm resulted in a nominal in-plane resolution of 24 μm × 24 μm.

of glucose, and of some amino acids, which can be correlated to the anatomy of the plant by superimposing the metabolite images and the high resolution image in Fig. 2. Since sucrose is the dominant carbohydrate in the phloem, we expected and found high sucrose concentrations in the vascular bundles (Fig. 3*A*). However, the two stereoisomers of glucose were mainly found in the pith parenchyma (α- and β-glucose; Fig. 3 *B* and *C*). The observation that the cortex parenchyma is rich in sucrose, whereas the pith parenchyma is rich in glucose, was unexpected. The biological significance of this complementary spatial distribution must be speculative at this early stage: the prevalence of hexoses in the pith parenchyma might contribute to a sufficiently high osmotic potential serving to maintain the turgor of the hypocotyl. The different locations of sucrose and glucose may have important consequences for the conflicting models of extension growth of shoots.

Glutamine is the major amino acid in the phloem sap (17) and is considered to be the main nitrogen carrier in castor bean seedlings. In the metabolite images, glutamine/glutamate occurs mostly in the cortex and the vascular bundles (Fig. 3*E*), whereas lysine (Fig. 3*F*) and arginine (Fig. 3*G*) are prevalent in the vascular bundles only. In earlier studies analyzing phloem exudate, it was found that the arginine concentration in the sieve tubes is not higher than in extracts of hypocotyl tissue (17). However, our CPI results reveal a prevalence of arginine in the vascular bundles. This may indicate an enrichment of arginine in the bundle parenchyma cells. The metabolic image of valine (Fig. 3*H*) shows a distribution that is restricted to the cortex parenchyma outside the vascular bundles. Within our limit of sensitivity, we could not observe any valine cross-peak in the vascular bundles.

DISCUSSION

The CPI experiment enables the observation of molecules that are typically accessible by NMR spectroscopy in the liquid state. These molecules must be relatively small and mobile, because any motional restriction of the spins results in a

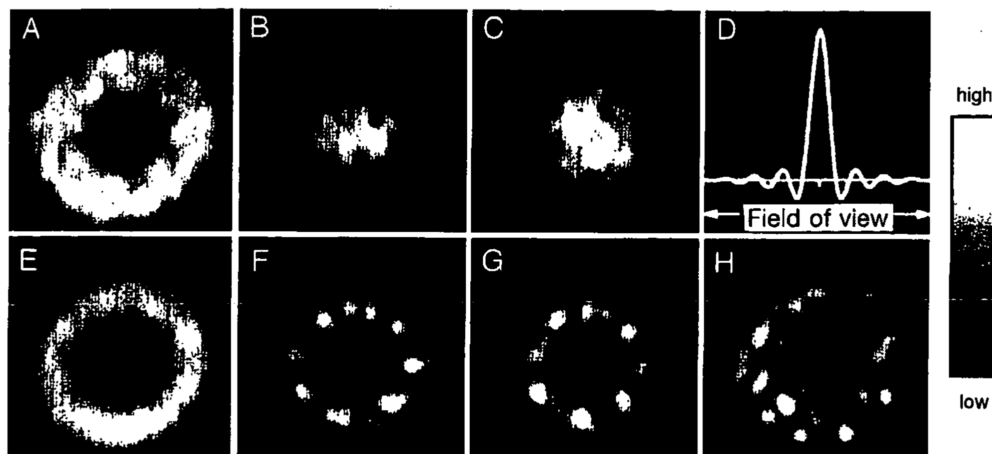

FIG. 3. Metabolic images obtained in a CPI experiment, in the same cross section of the hypocotyl as shown in the NMR image in Fig. 2. These images were obtained by selecting individual cross-peaks in the correlation spectra and by displaying their spatial distribution. The gray scale was adjusted individually for each image to obtain maximal contrast. (*A*) The distribution of the sucrose cross-peak shows high intensity in the vascular bundles, while the signal is lower in the cortex parenchyma. In the pith parenchyma, the sucrose intensity was low and decreasing towards the center, confirming the results of earlier CSI experiments (10). Because of the gray scaling, this gradient cannot be seen in *A*, but it clearly appears when plotting intensity profiles. The distribution of the signals corresponding to α- and β-glucose (*B* and *C*, respectively) reveals high intensity in the pith parenchyma. The signal of glutamine/glutamate (*E*) appears as a bright ring covering the cortex parenchyma and the vascular bundles. Despite their low concentration, lysine (*F*) and arginine (*G*) can be observed in the vascular bundles. Valine (*H*) was only found in the cortex parenchyma but not (within the limits of sensitivity) in the vascular bundles. Experimental details: in the four-dimensional CPI experiment, 16 × 16 localized correlation spectra were acquired in a field of view of 6 mm × 6 mm. The selection of a 4-mm slice along the hypocotyl resulted in a nominal volume of 560 nl for each image voxel. After Fourier transformation, the integrated intensity of individual cross-peaks was extracted, Fourier-interpolated to obtain a 256 × 256 image matrix, and scaled individually. The quality of the spatial localization can be assessed by the point spread function shown in *D*.

broadening of the resonance frequencies and a shortening of the transverse relaxation time, thereby impeding detection in the CPI experiment. This, in turn, will hinder the detection of molecules that are bound to membranes and may also reduce the "NMR visibility" of compounds fixed in larger storage molecules. Another limitation of NMR spectroscopy is its inherently low sensitivity. The lower limit whereby concentration can still be detected is determined—among other parameters—by the strength of the main magnetic field of the spectrometer, by the duration of the experiment, and by the size of the voxels in the metabolic image—i.e., the spatial resolution. The higher the magnetic field or the longer the experiment or the larger the voxels, the lower is the detectable concentration limit. In our experiments at 11.7 Tesla, with an experimental duration of 4 h and 33 min and a voxel size of 560 nl, we were able to observe metabolite concentrations to the order of 10 mM. Increasing the experimental duration may be used to improve spatial resolution or to increase the sensitivity of the CPI experiment to detect lower concentrated metabolites. Finally, the size of the experimental arrangement including the regulation of environmental parameters such as temperature, humidity, and light is limited by the space available in the NMR instrument; the bore size of our instrument was 89 mm.

The CPI experiment makes possible the identification of a large variety of chemical compounds and the measurement of their spatial distribution within the plant. The CPI experiment even enables one to distinguish between various stereoisomers. It has been shown that transport and metabolic reactions can depend strongly on the stereospecificity (15, 18), but until now the stereo configuration of basic sugars in the plant cells has not been known. In the same experimental setup, NMR images with high spatial resolution can be obtained, which allow one to correlate the measured distribution of metabolites with the anatomy of the plant. The main advantage of NMR methodology lies in its noninvasiveness. The experiments may be conducted repeatedly on the same plant and can monitor dynamic changes of the metabolites, typically in response to changing experimental parameters. For instance, the osmoregulation of cell turgor by changing hexose concentrations could be continuously monitored on an individual plant. CPI could thus become a versatile tool in studies of the reaction of plants to environmental stress. The combination of the molecular information accessible by multidimensional spectroscopic techniques and of the spatial information obtained by CSI may open a wide field of research in plant physiology.

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